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EDITORIAL

Pancreatic cancer: Translational research aspects and clinical implications

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Abstract

Despite improvements in surgical techniques and adjuvant chemotherapy, the overall mortality rates in pancreatic cancer have generally remained relatively unchanged and the 5-year survival rate is actually below 2%. This paper will address the importance of achieving an early diagnosis and identifying markers for prognosis and response to therapy such as genes, proteins, microRNAs or epigenetic modifications. However, there are still major hurdles when translating investigational biomarkers into routine clinical practice. Furthermore, novel ways of secondary screening in high-risk individuals, such as artificial neural networks and modern imaging, will be discussed. Drug resistance is ubiquitous in pancreatic cancer. Several mechanisms of drug resistance have already been revealed, including human equilibrative nucleoside transporter-1 status, multidrug resistance proteins, aberrant signaling pathways, microRNAs, stromal influence, epithelial-mesenchymal transition-type cells and recently the presence of cancer stem cells/cancer-initiating cells. These factors must be considered when developing more customized types of intervention ("personalized medicine"). In the future, multifunctional nanoparticles that combine a specific targeting agent, an imaging probe, a cell-penetrating agent, a biocompatible polymer and an anti-cancer drug may become valuable for the management of patients with pancreatic cancer.

Key words: Pancreatic cancer; Biomarkers; Imaging; Artificial neural networks; Nanomedicine; Personalized medicine

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INTRODUCTION

Pancreatic cancer has an approximate incidence of 11.4/100 000 inhabitants per year, and is recognized as the fourth cause of cancer-related death, with an overall 5-year survival of less than 1%-2%[1-3]. Total costs, including care-related costs and loss of production (due especially to premature death) related to pancreatic cancer in Sweden in the year 2009 were 86-93 million euros (population 9.1 million), corresponding to a society cost in the West of up to 10 million euros per 1 million inhabitants per year[4]. Smoking and also family history (in about 5%-10% of cases) are established risk factors for the development of pancreatic cancer[5]. There is a weaker positive associa-

tion for other factors including obesity, diabetes mellitus, chronic pancreatitis, ABO genotype, race, periodontal disease, occupational exposures, dietary factors, *Helicobacter pylori* and gallstones[5,6]. It is to be stated that the median age at diagnosis is in general 66-68 years[7], though early onset pancreatic cancer, i.e., occurring prior to 50 years of age, accounts for less than 6% of patients and is associated with more advanced disease at presentation and a tendency for shorter overall survival[8]. Gender-specific differences in the incidence of pancreatic cancer have been observed, including higher rates in males[9].

Chemotherapy and to a lesser extent, radiotherapy, have emerged as valuable adjuncts to the management of pancreatic cancer. A few studies reported that "marginally resectable" pancreatic tumors shrink after radiochemotherapy and may become resectable[10-12]. Neoadjuvant treatment of resectable pancreatic cancer is associated with fewer positive lymph nodes and increased survival (median 34 mo *vs* 19 mo, $P = 0.03$)[13]. In the ESPAC-1 study, 6 mo of postoperative 5-fluorouracil (5-FU) and folinic acid (FA) increased median survival from 14 mo to 19.7 mo, but there was no effect provided by radiochemotherapy[14]. Long-term follow-up after adjuvant chemotherapy demonstrated even better results with a median 21-23 mo survival following adjuvant chemotherapy *vs* 8-16 mo for observation[15,16]. The validity of gemcitabine as an adjuvant agent has been confirmed[17]. The ESPAC-3 study reported similar outcomes between 5-FU and FA *vs* gemcitabine ($n = 1088$)[18]. In unresectable pancreatic cancer, most regimens are also gemcitabine-based. The use of gemcitabine has increased median survival from 3-4 mo to 5.5-7 mo[19-21]. Recently, FOLFIRINOX (oxaliplatin, irinotecan, leucovorin, fluorouracil) surpassed the effectiveness of gemcitabine by showing longer survival (11.1 mo *vs* 6.8 mo; $P < 0.001$)[22]. The utilization of molecular targeted treatment in pancreatic cancer outside of clinical trials has been limited. Erlotinib provided a modest survival benefit in advanced pancreatic cancer when used in combination with gemcitabine (6.2 mo *vs* 5.9 mo)[23], but due to increased side-effects and increased costs it has not received wide clinical acceptance.

This paper will focus on clinical and molecular aspects of pancreatic cancer, discussing novel ways to improve early detection and prognostic prediction, as well as the design of future targeted therapy, which is imperative in this era of personalized medicine.

MOLECULAR PATHOGENESIS

Pancreatic ductal adenocarcinoma (PDAC) is believed to arise from precursor lesions that develop into invasive carcinoma through a multistep carcinogenic process. Pancreatic intraepithelial neoplasia (PanIN) is the most common preneoplastic lesion in patients with pancreatic cancer, being observed in approximately 80% of cases[24]. Other precursor lesions of PDAC are intraductal papillary mucinous neoplasms (IPMN) and mucinous cystic neoplasms (MCN). The *KRAS* oncogene is the most commonly altered gene in pancreatic cancer. Inactivation of the tumor-suppressor genes *CDKN2A*, *TP53*, *DPC4* and *BRCA2*, as well as chromosomal losses, gene amplifications and telomere shortening have also been observed[6]. Reactivation of developmental pathways, such as hedgehog, notch and wnt/β-catenin, may be crucial for the development of PDAC[25]. In addition to genetic alterations, many lines of evidence indicate that epigenetic changes play a role in pancreatic carcinogenesis. DNA methylation and histone modification frequently alter gene function without changing the DNA sequence, and have the potential to be used as diagnostic markers in pancreatic cancer[26]. MicroRNAs are non-coding segments of RNA that can regulate gene expression. Aberrant expression of microRNAs contributes to tumor progression and has been associated with drug resistance[27].

Because all three precursor lesions of PDAC possess ductal characteristics, it has been suggested that the lesions develop from ductal cells. However, the study of mouse models of pancreatic cancer has broadened the current understanding of pancreatic carcinogenesis by showing other cells to be the cancer-initiating cells. Differentiated acinar cells have been shown to cause PanIN and pancreatic cancer following activation of KRAS *in vivo*[28-30]. Moreover, insulin-positive endocrine cells and PDX1-expressing cells have been demonstrated to induce PDAC[31]. It should be noted that the cell of origin, in which tumorigenesis is initiated, could be different from the cancer stem cell, which propagates the tumor[32]. Identification of cells of origin in PDAC may allow earlier detection of malignancy and better preventive and treatment tools.

Crosstalk tumor-stroma

Desmoplasia is a characteristic feature of pancreatic cancer and the stromal compartment has been considered to be a physical barrier for drug delivery[33]. The pancreatic stellate cell (PSC) has a key role in stroma formation. In addition to endogenous quiescent PSCs, bone marrow may also contribute to the population of activated PSCs[34]. PSCs are involved in tumor growth, invasion, metastasis and resistance to radiochemotherapy[35-37]. Furthermore, PSCs accompany cancer cells to distant metastatic sites, stimulate angiogenesis and have the capacity to migrate over the endothelial barrier to and from blood vessels[38]. A limited number of studies have attempted to block PSC activity in the setting of pancreatic cancer. For example, halofuginine, a smad3-phosphorylation-inhibitor, reduces PSC activation and prevents pancreatic xenograft tumor development[39]. Retinoic acid can also inhibit PSC activity and reduces wnt-β-catenin signaling in cancer cells and their invasive ability[40]. Key signaling pathways between PSCs and cancer cells have been identified and involve e.g., sonic hedgehog, galectins, endothelins and platelet-derived growth factor[35], which thereby represent potential therapeutic targets.

Cancer stem cells and epithelial-mesenchymal transition

Pancreatic cancer stem cells constitute a minority of cancer cells (1%-5%) and have the ability to self-renew, and

are resistant to chemotherapy and radiation[41]. They are characterized by several surface markers including CD44, CD24, epithelial specific antigen, aldehyde dehydrogenase, CD133 and CXCR4[42]. Furthermore, it has been observed that pancreatic cancer cells that were cultured in gemcitabine demonstrated characteristics of epithelial-mesenchymal transition (EMT)[43]. They also showed increased expression of cell surface proteins associated with cancer stem cells. In pancreatic cancer xenografts, radiation or gemcitabine therapy leads to enrichment of the EMT cells[44]. Wnt, notch and hedgehog are important signaling events in cancer stem cells, and can become novel therapeutic targets[41]. Ongoing clinical trials are currently investigating PRI-724 (inhibitor of wnt), MK-0752 (inhibitor of notch) and GDC-0449 (inhibitor of hedgehog) in patients with advanced pancreatic cancer (www.clinicaltrials.gov). Future therapeutic strategies may need to combine targeting of cancer stem cells and EMT cells with the targeting of other cells in the microenvironment, e.g., stromal cells, in order to achieve maximal benefit.

Pro-inflammatory response

Inflammation is closely related to the development and progression of pancreatic cancer, and molecular factors such as STAT3 have been suggested to play a key role in creating a pro-inflammatory tumor microenvironment[45]. Clinical studies have shown that a pro-inflammatory response is both prognostically negative and promotes tumor proliferation[46]. Inflammatory factors may also contribute to the profound weight loss and cancer cachexia frequently seen in pancreatic cancer[47]. Elucidation of the mechanisms underlying the crosstalk between inflammation, cancer and stroma may improve the management of pancreatic cancer, as a frequent desmoplastic reaction is noted.

Chemoresistance

Gemcitabine has represented the first-line of chemotherapeutic agents in pancreatic cancer. A frequent problem, though, is drug resistance and lack of response to therapy given. Nucleoside transporters, such as human equilibrative nucleoside transporter-1 (hENT-1), appear to regulate the intracellular uptake of gemcitabine[48]. One of the proposed mechanisms of chemoresistance is a reduction in hENT-1 expression. Determination of hENT-1 status at the time of cancer diagnosis, and also modifications of gemcitabine in order to bypass the nucleoside receptor, may represent novel types of targeted approaches in the management of patients with pancreatic cancer[48]. Multidrug resistance (MDR) proteins including ABC-transporters have also been implicated in drug resistance in pancreatic cancer and limit the efficacy of gemcitabine[49]. Another mechanism that contributes to chemoresistance is the tumor microenvironment surrounding the cancer cells, including cancer stem cells, EMT cells and stellate cells. Furthermore, the hypoxic stroma could be a physical barrier preventing chemotherapeutic drugs from reaching pancreatic cancer cells, and depletion of the stroma could enhance cancer drug delivery[33]. Aberrant signaling pathways also have a role in drug resistance. The PI3K/Akt signaling pathway is commonly overactive in pancreatic cancer. PI3K stimulates proliferation and confers chemoresistance[50]. MicroRNAs have received increased attention in recent years. Targeting of microRNAs may help overcome drug resistance in pancreatic caner and improve clinical outcome[27].

BIOMARKERS

Biomarkers can be applied in several areas of disease management including diagnosis, prognosis, staging and prediction and monitoring of therapeutic response. The different types of biomarkers include genes, proteins, metabolites, microRNAs and epigenetic modifications. CA 19-9 has some value for detection of recurrent disease[51], but so far no other biomarker is recommended for routine clinical use in pancreatic cancer. Recently, a seven-gene panel was identified as being differentially expressed between pancreatic cancer (n = 36) and normal samples (n = 19)[52]. Validation using two blood-based biomarkers from this panel, tenascin C and tissue factor pathway inhibitor, yielded a combined area under the curve (AUC) of 0.88 and, with addition of CA19-9, a combined AUC for the three-gene panel of 0.99 with 100% specificity at 90% sensitivity and 97% sensitivity at 90% specificity.

Proteomic profiling of pancreatic cancer serum has been promising. Most studies have used surface enhanced laser desorption (SELDI) or matrix assisted laser desorption/ionization (MALDI) yielding a sensitivity in the range of 78% to 100% and a specificity between 74% and 100%[53]. Immunohistochemistry (IHC) is the most practical method for evaluating protein expression changes in histopathology. It can be combined with tissue microarray technology to allow rapid testing of immunohistochemical markers on many tumors in a single experiment. During the past decade, a multitude of immunohistochemical biomarkers that are potentially involved in pancreatic carcinogenesis and drug responsiveness have been studied for their prognostic and predictive value, but none of them have yet proved to be sufficiently useful for use in routine clinical practice[54]. Apart from the tumor compartment, stromal tissue may also be analyzed and it has been discovered that stromal secreted protein acidic and rich in cysteine has been associated with outcome in pancreatic cancer[55]. A panel of IHC markers may prove clinically valuable in the future. Furthermore, metabolomic studies of pancreatic cancer are promising and may be useful in identifying benign from malignant conditions[56-58]. MicroRNA is a new class of biomarkers. Aberrant expression of miRNA-21 and miRNA-34a has been associated with survival in resectable pancreatic cancer[59]. Epigenetic changes, such as histone modification, may be used as novel biomarkers in pancreatic cancer[60].

Although a multitude of investigational biomarkers have been identified, translation into routine clinical

practice has been difficult. To improve methodological reporting several guidelines have been developed. For diagnostic biomarkers, the STAndards for Reporting Diagnostic accuracy (STARD) guidelines are available[61]. For prognostic studies, the REporting recommendations for tumor MARKer prognostic studies (REMARK) guidelines are available[62]. The process of translating biomarkers is complex and the path from discovery to clinical application may be long and arduous. The effective demonstration of clinical utility of the biomarker will remain the key to its gaining widespread acceptance, but regulatory issues and budgetary constraints of the biomarker industry remain major challenges[63].

IMAGING

The detection of precursor lesions of pancreatic cancer would be a key factor in improving the prognosis. Non-invasive imaging techniques such as ultrasound, computed tomography and magnetic resonance imaging do not accurately identify PanINs. Positron emission tomography (PET) is a functional imaging modality that utilizes the principle that metabolic alterations in tumors occur prior to notable morphological alterations. The radioactive tracer ^{18}F-fluorodeoxyglucose (FDG) has been used extensively for PET imaging of malignant tumors. Malignant tissue has increased glucose metabolism as compared to its surrounding tissue, which leads to focal FDG-uptake visualized by PET. PET/CT has come to play an increasing role in pancreatic cancer, due to the ability to accurately detect small primary pancreatic lesions and distant metastases, as well as recurrences following surgery[64-67]. It has been shown that an elevated glucose metabolism occurs already in precursor lesions of pancreatic cancer, with the opportunity of detecting these changes with PET/CT, and thus improving diagnosis and outcome[68]. Eser *et al*[69] recently described a technique that could improve diagnosis and also grading of PanINs using *in vivo* molecular imaging based on cathepsins.

PANCREATIC CANCER AND DIABETES MELLITUS

Up to 80% of patients with pancreatic cancer have diabetes mellitus or pathologic glucose tolerance test at diagnosis[70]. Long-standing type Ⅱ diabetes is a predisposing factor for pancreatic cancer, while new-onset diabetes may indicate subclinical cancer[71]. The molecular mechanisms linking long-standing diabetes to pancreatic cancer are incompletely understood. Diabetes may promote the neoplastic process by several mechanisms including hyperinsulinemia (endogenous or exogenous), hyperglycemia and chronic inflammation[72]. The insulin and insulin-like growth factor (IGF) receptors are frequently expressed in pancreatic cancer and contribute to neoplastic growth and progression[73]. The administration of the anti-diabetic agent metformin may reduce the incidence of pancreatic cancer in patients with type Ⅱ diabetes[74,75]. In xenograft models, metformin inhibits the growth of pancreatic cancer cells by interfering with insulin/IGF-1 receptor and G-protein-coupled receptor signaling[76]. In addition, metformin can inhibit tumor growth by inactivating cancer stem cell-like cells[77]. Studies have sought to elucidate molecular alterations that link diabetes and cancer, and one such molecular connection could be TCF7L2 (T-cell factor 7-like 2) and p53[78]. In a recently published case-control study, rs780094 was selected as one of 10 diabetes-associated single-nucleotide polymorphisms related to increased pancreatic cancer risk[79]. However, diabetes in pancreatic cancer is mostly new-onset, i.e., occurring 24 mo prior to cancer diagnosis, and is likely related to secondary effects from the tumor, which is supported by the observation that glucose metabolism is improved following tumor resection[71,80]. Although the exact mechanisms behind pancreatic cancer-induced diabetes are yet to be disclosed, there is ample evidence for a tumor-derived influence on glucose metabolism, leading to disturbed β-cell function, peripheral insulin resistance, hyperglycemia and finally diabetes mellitus[70].

SCREENING

Pancreatic cancer develops over a long time span, providing a strong rationale for developing techniques for early detection. It may take ten years or more between the initial mutation and first non-metastatic tumor cell, and another five years for the development of metastatic capacity and death after an additional two years[81]. This implies a therapeutic window of opportunity for both early diagnosis and treatment. Chromothripsis is a new concept that involves the simultaneous acquisition of multiple mutations in a single catastrophic event. This phenomenon may be present in 2%-3% of all human cancers, but the incidence may be higher in certain tumors, such as osteosarcomas and chordomas[82].

Patients with pancreatic cancer usually have generic symptoms and are often difficult to diagnose at an early stage. There are several risk groups where secondary screening for pancreatic cancer may be appropriate, e.g., patients with heredity, IPMN, or new-onset diabetes mellitus[70,83-85]. Distinguishing pancreatic cancer-associated diabetes from the more common general type 2 diabetes may identify individuals with a potentially resectable pancreatic cancer[70-71]. Huang *et al*[86] identified vanin-1 and matrix metalloproteinase 9 as useful biomarkers for the discrimination of pancreatic cancer-associated diabetes from type Ⅱ diabetes.

Artificial neural networks represent non-linear pattern recognition techniques that simulate the analytic processes of the human brain. They have been utilized in complex medical decision-making, including diagnosis, prognosis and risk stratification[87]. A major benefit of these networks is the ability to recognize complex relationships between input and output data that may be hidden to conventional statistical methods. Initial reports on the use of artificial neural networks combined with proteomic

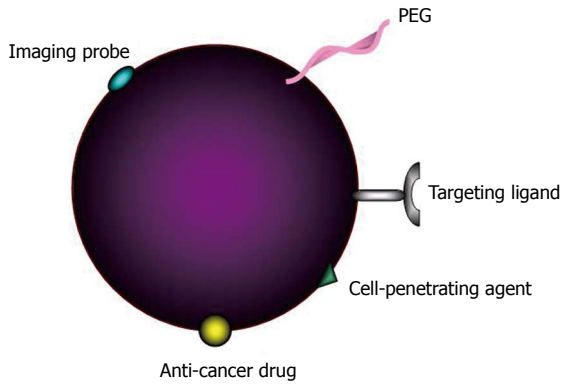

Figure 1 Multifunctional nanoparticle. PEG: Polyethylene glycol.

data have provided promising results concerning the detection of pancreatic cancer[88]. The future application of artificial neural networks based on parameters including age, smoking, heredity, chronic pancreatitis, new-onset diabetes mellitus, biomarkers and imaging findings imply promise for early detection of pancreatic cancer, and may be used as screening tools.

NANOMEDICINE

Nanomedicine is defined as the application of nanotechnology to medicine. Nanoparticles are in the range of 1-100 nm. Examples of nanoparticles include liposomes (phospholipid vesicles), dendrimers (synthetic polymers), carbon nanotubes (fullerene), quantum dots (colloidal fluorescent semiconductor nanocrystals), magnetic nanoparticles (spherical nanocrystals with a Fe^{2+} and Fe^{3+} core) and gold nanoparticles (metallic nanoparticles). The application of nanoparticles in medicine include e.g., diagnostics, imaging and drug delivery.

Nanoparticles enable refined diagnostics at the level of single cells and molecules. For example, magnetic nanoparticles have been coupled with molecular targeting ligands to improve imaging of early pancreatic tumors *in vivo*[89]. Quantum dots conjugated with RGD peptides have been reported for *in vivo* imaging of pancreatic tumor vasculature[90]. Drug resistance is a recognized challenge in pancreatic cancer. Gemcitabine-squalene obtained by covalently coupling gemcitabine at the 4-amino group with squalene, a natural lipid, has been shown to make tumor cells more sensitive to gemcitabine[91]. Recently, polymeric nanoparticles encapsulating hedgehog-inhibitors or curcumin have been produced that inhibit the growth of orthotopic pancreatic cancer xenografts[92,93]. Gold nanoparticles have been utilized to induce intracellular hyperthermia in a murine model of pancreatic cancer after radiofrequency field exposure[94]. An ongoing phase I study (NCT00968604) of advanced pancreatic cancer is currently investigating the effects of intravenous injection of the liposome nanoparticle BikDD, which contains a pro-apoptotic agent. Several nanoparticle-based anticancer drugs are already on the market, e.g., Abraxane® (albumin-bound paclitaxel), Myocet® (liposomal doxorubicin) and Oncaspar® (PEG-L-asparaginase)[95].

While monofunctional nanoparticles only carry out one function, multifunctional nanoparticles have the ability to perform several tasks. Multifunctional nanocarriers using a specific targeting agent, an imaging probe, a cell-penetrating agent such as TAT peptide, a biocompatible polymer such as polyethylene glycol (PEG) and an anti-cancer drug, may result in effective tumor destruction with minimal toxicity (Figure 1).

CONCLUSION

Pancreatic cancer is a condition with an almost total lethal outcome. Despite advancement in surgical techniques and adjuvant treatment, the prognosis has only marginally improved. Novel therapeutic interventions have been tested but with limited effect. Research should continue to focus on biomarkers for early diagnosis, prognosis and prediction and monitoring of therapeutic response. Screening of high-risk individuals using novel approaches such as artificial neural networks could be considered. Mechanisms of chemoresistance have been elucidated, including hENT-1 status, MDR proteins, aberrant signaling pathways, microRNAs and micro-environmental factors, which should underlie future development of targeted therapy. The identification of cancer-initiating cells represents a fundamental shift in our understanding of the intrinsic drug resistance of pancreatic cancer. Multifunctional nanoparticles have the potential to combine imaging, diagnosis and therapy in a single vehicle. It is expected that nanomedicine will have a prominent role in the quest for a successful therapy for this recalcitrant disease.

REFERENCES

1 **Gudjonsson B**. Cancer of the pancreas. 50 years of surgery. *Cancer* 1987; **60**: 2284-2303
2 **Gudjonsson B**. Critical look at resection for pancreatic cancer. *Lancet* 1996; **348**: 1676
3 **Bray F**, Sankila R, Ferlay J, Parkin DM. Estimates of cancer incidence and mortality in Europe in 1995. *Eur J Cancer* 2002; **38**: 99-166
4 **Tingstedt B**, Andersson E, Flink A, Bolin K, Lindgren B, Andersson R. Pancreatic cancer, healthcare cost, and loss of productivity: a register-based approach. *World J Surg* 2011; **35**: 2298-2305
5 **Hart AR**, Kennedy H, Harvey I. Pancreatic cancer: a review of the evidence on causation. *Clin Gastroenterol Hepatol* 2008; **6**: 275-282
6 **Vincent A**, Herman J, Schulick R, Hruban RH, Goggins M. Pancreatic cancer. *Lancet* 2011; **378**: 607-620
7 Cancer: principles and practice of oncology. 6th ed. DeVita VT Jr, Hellman S, Rosenberg SA, editors. Philadelphia, PA: Lippincott Williams and Wilkins, 2001: 1126-1161
8 **Tingstedt B,** Weitkämper C, Andersson R. Early onset pancreatic cancer-comparison against matched controls. *Ann Gastronterol* 2011; **24**: 206-212
9 **Maisonneuve P**, Lowenfels AB. Epidemiology of pancreatic cancer: an update. *Dig Dis* 2010; **28**: 645-656
10 **Arnoletti JP**, Hoffman JP, Ross EA, Kagan SA, Meropol NJ, Freedman G, Eisenberg B. Preoperative chemoradiation in the management of adenocarcinoma of the body of the pancreas. *Am Surg* 2002; **68**: 330-335; discussion 335

11 **Stokes JB**, Nolan NJ, Stelow EB, Walters DM, Weiss GR, de Lange EE, Rich TA, Adams RB, Bauer TW. Preoperative capecitabine and concurrent radiation for borderline resectable pancreatic cancer. *Ann Surg Oncol* 2011; **18**: 619-627
12 **Mehta VK**, Fisher G, Ford JA, Poen JC, Vierra MA, Oberhelman H, Niederhuber J, Bastidas JA. Preoperative chemoradiation for marginally resectable adenocarcinoma of the pancreas. *J Gastrointest Surg* 2001; **5**: 27-35
13 **Artinyan A**, Anaya DA, McKenzie S, Ellenhorn JD, Kim J. Neoadjuvant therapy is associated with improved survival in resectable pancreatic adenocarcinoma. *Cancer* 2011; **117**: 2044-2049
14 **Neoptolemos JP**, Dunn JA, Stocken DD, Almond J, Link K, Beger H, Bassi C, Falconi M, Pederzoli P, Dervenis C, Fernandez-Cruz L, Lacaine F, Pap A, Spooner D, Kerr DJ, Friess H, Büchler MW. Adjuvant chemoradiotherapy and chemotherapy in resectable pancreatic cancer: a randomised controlled trial. *Lancet* 2001; **358**: 1576-1585
15 **Neoptolemos JP**, Stocken DD, Tudur Smith C, Bassi C, Ghaneh P, Owen E, Moore M, Padbury R, Doi R, Smith D, Büchler MW. Adjuvant 5-fluorouracil and folinic acid vs observation for pancreatic cancer: composite data from the ESPAC-1 and -3(v1) trials. *Br J Cancer* 2009; **100**: 246-250
16 **Choti MA**. Adjuvant therapy for pancreatic cancer--the debate continues. *N Engl J Med* 2004; **350**: 1249-1251
17 **Oettle H**, Post S, Neuhaus P, Gellert K, Langrehr J, Ridwelski K, Schramm H, Fahlke J, Zuelke C, Burkart C, Gutberlet K, Kettner E, Schmalenberg H, Weigang-Koehler K, Bechstein WO, Niedergethmann M, Schmidt-Wolf I, Roll L, Doerken B, Riess H. Adjuvant chemotherapy with gemcitabine vs observation in patients undergoing curative-intent resection of pancreatic cancer: a randomized controlled trial. *JAMA* 2007; **297**: 267-277
18 **Neoptolemos JP**, Stocken DD, Bassi C, Ghaneh P, Cunningham D, Goldstein D, Padbury R, Moore MJ, Gallinger S, Mariette C, Wente MN, Izbicki JR, Friess H, Lerch MM, Dervenis C, Oláh A, Butturini G, Doi R, Lind PA, Smith D, Valle JW, Palmer DH, Buckels JA, Thompson J, McKay CJ, Rawcliffe CL, Büchler MW. Adjuvant chemotherapy with fluorouracil plus folinic acid vs gemcitabine following pancreatic cancer resection: a randomized controlled trial. *JAMA* 2010; **304**: 1073-1081
19 **Berlin JD**, Catalano P, Thomas JP, Kugler JW, Haller DG, Benson AB. Phase III study of gemcitabine in combination with fluorouracil versus gemcitabine alone in patients with advanced pancreatic carcinoma: Eastern Cooperative Oncology Group Trial E2297. *J Clin Oncol* 2002; **20**: 3270-3275
20 **Burris HA**, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, Cripps MC, Portenoy RK, Storniolo AM, Tarassoff P, Nelson R, Dorr FA, Stephens CD, Von Hoff DD. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. *J Clin Oncol* 1997; **15**: 2403-2413
21 **Moore MJ,** Hamm J, Eisenberg P, Dagenais M, Hagan K, Fields A, Greenberg B, Schwartz B, Ottaway J, Zee B, Seymour L. A Comparison Between Gemcitabine (GEM) and the Matrix Metalloproteinase (MMP) Inhibitor BAY12-9566 (9566) in Patients (PTS) with Advanced Pancreatic Cancer. *Proc Am Soc Clin Oncol* 2000; **19**: A930
22 **Conroy T**, Desseigne F, Ychou M, Bouché O, Guimbaud R, Bécouarn Y, Adenis A, Raoul JL, Gourgou-Bourgade S, de la Fouchardière C, Bennouna J, Bachet JB, Khemissa-Akouz F, Péré-Vergé D, Delbaldo C, Assenat E, Chauffert B, Michel P, Montoto-Grillot C, Ducreux M. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N Engl J Med* 2011; **364**: 1817-1825
23 **Moore MJ**, Goldstein D, Hamm J, Figer A, Hecht JR, Gallinger S, Au HJ, Murawa P, Walde D, Wolff RA, Campos D, Lim R, Ding K, Clark G, Voskoglou-Nomikos T, Ptasynski M, Parulekar W. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. *J Clin Oncol* 2007; **25**: 1960-1966
24 **Hruban RH**, Maitra A, Goggins M. Update on pancreatic intraepithelial neoplasia. *Int J Clin Exp Pathol* 2008; **1**: 306-316
25 **Rhim AD**, Stanger BZ. Molecular biology of pancreatic ductal adenocarcinoma progression: aberrant activation of developmental pathways. *Prog Mol Biol Transl Sci* 2010; **97**: 41-78
26 **Omura N**, Goggins M. Epigenetics and epigenetic alterations in pancreatic cancer. *Int J Clin Exp Pathol* 2009; **2**: 310-326
27 **Frampton AE**, Krell J, Jacob J, Stebbing J, Jiao LR, Castellano L. microRNAs as markers of survival and chemoresistance in pancreatic ductal adenocarcinoma. *Expert Rev Anticancer Ther* 2011; **11**: 1837-1842
28 **De La O JP**, Emerson LL, Goodman JL, Froebe SC, Illum BE, Curtis AB, Murtaugh LC. Notch and Kras reprogram pancreatic acinar cells to ductal intraepithelial neoplasia. *Proc Natl Acad Sci USA* 2008; **105**: 18907-18912
29 **Habbe N**, Shi G, Meguid RA, Fendrich V, Esni F, Chen H, Feldmann G, Stoffers DA, Konieczny SF, Leach SD, Maitra A. Spontaneous induction of murine pancreatic intraepithelial neoplasia (mPanIN) by acinar cell targeting of oncogenic Kras in adult mice. *Proc Natl Acad Sci USA* 2008; **105**: 18913-18918
30 **Ji B**, Tsou L, Wang H, Gaiser S, Chang DZ, Daniluk J, Bi Y, Grote T, Longnecker DS, Logsdon CD. Ras activity levels control the development of pancreatic diseases. *Gastroenterology* 2009; **137**: 1072-1082
31 **Gidekel Friedlander SY**, Chu GC, Snyder EL, Girnius N, Dibelius G, Crowley D, Vasile E, DePinho RA, Jacks T. Context-dependent transformation of adult pancreatic cells by oncogenic K-Ras. *Cancer Cell* 2009; **16**: 379-389
32 **Visvader JE**. Cells of origin in cancer. *Nature* 2011; **469**: 314-322
33 **Olive KP**, Jacobetz MA, Davidson CJ, Gopinathan A, McIntyre D, Honess D, Madhu B, Goldgraben MA, Caldwell ME, Allard D, Frese KK, Denicola G, Feig C, Combs C, Winter SP, Ireland-Zecchini H, Reichelt S, Howat WJ, Chang A, Dhara M, Wang L, Rückert F, Grützmann R, Pilarsky C, Izeradjene K, Hingorani SR, Huang P, Davies SE, Plunkett W, Egorin M, Hruban RH, Whitebread N, McGovern K, Adams J, Iacobuzio-Donahue C, Griffiths J, Tuveson DA. Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. *Science* 2009; **324**: 1457-1461
34 **Scarlett CJ**, Colvin EK, Pinese M, Chang DK, Morey AL, Musgrove EA, Pajic M, Apte M, Henshall SM, Sutherland RL, Kench JG, Biankin AV. Recruitment and activation of pancreatic stellate cells from the bone marrow in pancreatic cancer: a model of tumor-host interaction. *PLoS One* 2011; **6**: e26088
35 **Dunér S**, Lopatko Lindman J, Ansari D, Gundewar C, Andersson R. Pancreatic cancer: the role of pancreatic stellate cells in tumor progression. *Pancreatology* 2010; **10**: 673-681
36 **Hwang RF**, Moore T, Arumugam T, Ramachandran V, Amos KD, Rivera A, Ji B, Evans DB, Logsdon CD. Cancer-associated stromal fibroblasts promote pancreatic tumor progression. *Cancer Res* 2008; **68**: 918-926
37 **Mantoni TS**, Lunardi S, Al-Assar O, Masamune A, Brunner TB. Pancreatic stellate cells radioprotect pancreatic cancer cells through β1-integrin signaling. *Cancer Res* 2011; **71**: 3453-3458
38 **Xu Z**, Vonlaufen A, Phillips PA, Fiala-Beer E, Zhang X, Yang L, Biankin AV, Goldstein D, Pirola RC, Wilson JS, Apte MV. Role of pancreatic stellate cells in pancreatic cancer metastasis. *Am J Pathol* 2010; **177**: 2585-2596
39 **Spector I**, Honig H, Kawada N, Nagler A, Genin O, Pines M.

Inhibition of pancreatic stellate cell activation by halofuginone prevents pancreatic xenograft tumor development. *Pancreas* 2010; **39**: 1008-1015
40 **Froeling FE**, Feig C, Chelala C, Dobson R, Mein CE, Tuveson DA, Clevers H, Hart IR, Kocher HM. Retinoic acid-induced pancreatic stellate cell quiescence reduces paracrine Wnt-β-catenin signaling to slow tumor progression. *Gastroenterology* 2011; **141**: 1486-1497
41 **Hidalgo M**. Pancreatic cancer. *N Engl J Med* 2010; **362**: 1605-1617
42 **Bednar F**, Simeone DM. Pancreatic cancer stem cell biology and its therapeutic implications. *J Gastroenterol* 2011; **46**: 1345-1352
43 **Shah AN**, Summy JM, Zhang J, Park SI, Parikh NU, Gallick GE. Development and characterization of gemcitabine-resistant pancreatic tumor cells. *Ann Surg Oncol* 2007; **14**: 3629-3637
44 **Lee CJ**, Dosch J, Simeone DM. Pancreatic cancer stem cells. *J Clin Oncol* 2008; **26**: 2806-2812
45 **Li N**, Grivennikov SI, Karin M. The unholy trinity: inflammation, cytokines, and STAT3 shape the cancer microenvironment. *Cancer Cell* 2011; **19**: 429-431
46 **Tingstedt B**, Johansson P, Andersson B, Andersson R. Predictive factors in pancreatic ductal adenocarcinoma: role of the inflammatory response. *Scand J Gastroenterol* 2007; **42**: 754-759
47 **Martignoni ME**, Kunze P, Hildebrandt W, Künzli B, Berberat P, Giese T, Klöters O, Hammer J, Büchler MW, Giese NA, Friess H. Role of mononuclear cells and inflammatory cytokines in pancreatic cancer-related cachexia. *Clin Cancer Res* 2005; **11**: 5802-5808
48 **Andersson R**, Aho U, Nilsson BI, Peters GJ, Pastor-Anglada M, Rasch W, Sandvold ML. Gemcitabine chemoresistance in pancreatic cancer: molecular mechanisms and potential solutions. *Scand J Gastroenterol* 2009; **44**: 782-786
49 **Hagmann W**, Jesnowski R, Löhr JM. Interdependence of gemcitabine treatment, transporter expression, and resistance in human pancreatic carcinoma cells. *Neoplasia* 2010; **12**: 740-747
50 **Ng SSW MS**, Chow S, Hedley DW. Inhibition of phosphatidylinositide 3-kinase enhances gemcitabine-induced apoptosis in human pancreatic cancer cells. *Cancer Res* 2000; **60**: 5451-5455
51 **Melton SD**, Genta RM, Souza RF. Biomarkers and molecular diagnosis of gastrointestinal and pancreatic neoplasms. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 620-628
52 **Balasenthil S**, Chen N, Lott ST, Chen J, Carter J, Grizzle WE, Frazier ML, Sen S, Killary AM. A migration signature and plasma biomarker panel for pancreatic adenocarcinoma. *Cancer Prev Res* (*Phila*) 2011; **4**: 137-149
53 **Pawa N**, Wright JM, Arulampalam TH. Mass spectrometry based proteomic profiling for pancreatic cancer. *JOP* 2010; **11**: 423-426
54 **Ansari D**, Rosendahl A, Elebro J, Andersson R. Systematic review of immunohistochemical biomarkers to identify prognostic subgroups of patients with pancreatic cancer. *Br J Surg* 2011; **98**: 1041-1055
55 **Infante JR**, Matsubayashi H, Sato N, Tonascia J, Klein AP, Riall TA, Yeo C, Iacobuzio-Donahue C, Goggins M. Peritumoral fibroblast SPARC expression and patient outcome with resectable pancreatic adenocarcinoma. *J Clin Oncol* 2007; **25**: 319-325
56 **Bathe OF**, Shaykhutdinov R, Kopciuk K, Weljie AM, McKay A, Sutherland FR, Dixon E, Dunse N, Sotiropoulos D, Vogel HJ. Feasibility of identifying pancreatic cancer based on serum metabolomics. *Cancer Epidemiol Biomarkers Prev* 2011; **20**: 140-147
57 **Napoli C**, Sperandio N, Lawlor RT, Scarpa A, Molinari H, Assfalg M. Urine metabolic signature of pancreatic ductal adenocarcinoma by (1)h nuclear magnetic resonance: identification, mapping, and evolution. *J Proteome Res* 2012; **11**: 1274-1283
58 **OuYang D**, Xu J, Huang H, Chen Z. Metabolomic profiling of serum from human pancreatic cancer patients using 1H NMR spectroscopy and principal component analysis. *Appl Biochem Biotechnol* 2011; **165**: 148-154
59 **Jamieson NB**, Morran DC, Morton JP, Ali A, Dickson EJ, Carter CR, Sansom OJ, Evans TR, McKay CJ, Oien KA. MicroRNA Molecular Profiles Associated with Diagnosis, Clinicopathologic Criteria, and Overall Survival in Patients with Resectable Pancreatic Ductal Adenocarcinoma. *Clin Cancer Res* 2012; **18**: 534-545
60 **Manuyakorn A**, Paulus R, Farrell J, Dawson NA, Tze S, Cheung-Lau G, Hines OJ, Reber H, Seligson DB, Horvath S, Kurdistani SK, Guha C, Dawson DW. Cellular histone modification patterns predict prognosis and treatment response in resectable pancreatic adenocarcinoma: results from RTOG 9704. *J Clin Oncol* 2010; **28**: 1358-1365
61 **Bossuyt PM**, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, Lijmer JG, Moher D, Rennie D, de Vet HC. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. *Clin Biochem* 2003; **36**: 2-7
62 **McShane LM**, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM. REporting recommendations for tumour MARKer prognostic studies (REMARK). *Br J Cancer* 2005; **93**: 387-391
63 **Semenuk MA,** Veenstra TD, Georgevich G, Rochman M, Gusev Y, Haanes EJ, Bogunovic BM, Lebowitz M, Daraselia N, Schwartz EI. Translating cancer biomarker discoveries to clinical tests: what should be considered? *Rec Pat Biom* 2011; **1**: 222-240
64 **Jadvar H**, Fischman AJ. Evaluation of pancreatic carcinoma with FDG PET. *Abdom Imaging* 2001; **26**: 254-259
65 **Okano K**, Kakinoki K, Akamoto S, Hagiike M, Usuki H, Yamamoto Y, Nishiyama Y, Suzuki Y. 18F-fluorodeoxyglucose positron emission tomography in the diagnosis of small pancreatic cancer. *World J Gastroenterol* 2011; **17**: 231-235
66 **Ruf J**, Lopez Hänninen E, Oettle H, Plotkin M, Pelzer U, Stroszczynski C, Felix R, Amthauer H. Detection of recurrent pancreatic cancer: comparison of FDG-PET with CT/MRI. *Pancreatology* 2005; **5**: 266-272
67 **Andersson R**, Vagianos CE, Williamson RC. Preoperative staging and evaluation of resectability in pancreatic ductal adenocarcinoma. *HPB* (*Oxford*) 2004; **6**: 5-12
68 **Fendrich V**, Schneider R, Maitra A, Jacobsen ID, Opfermann T, Bartsch DK. Detection of precursor lesions of pancreatic adenocarcinoma in PET-CT in a genetically engineered mouse model of pancreatic cancer. *Neoplasia* 2011; **13**: 180-186
69 **Eser S**, Messer M, Eser P, von Werder A, Seidler B, Bajbouj M, Vogelmann R, Meining A, von Burstin J, Algül H, Pagel P, Schnieke AE, Esposito I, Schmid RM, Schneider G, Saur D. In vivo diagnosis of murine pancreatic intraepithelial neoplasia and early-stage pancreatic cancer by molecular imaging. *Proc Natl Acad Sci USA* 2011; **108**: 9945-9950
70 **Bartosch-Härlid A**, Andersson R. Diabetes mellitus in pancreatic cancer and the need for diagnosis of asymptomatic disease. *Pancreatology* 2010; **10**: 423-428
71 **Magruder JT**, Elahi D, Andersen DK. Diabetes and pancreatic cancer: chicken or egg? *Pancreas* 2011; **40**: 339-351
72 **Giovannucci E**, Harlan DM, Archer MC, Bergenstal RM, Gapstur SM, Habel LA, Pollak M, Regensteiner JG, Yee D. Diabetes and cancer: a consensus report. *CA Cancer J Clin* 2010; **60**: 207-221
73 **Li D**. Diabetes and pancreatic cancer. *Mol Carcinog* 2012; **51**: 64-74
74 **Lee MS**, Hsu CC, Wahlqvist ML, Tsai HN, Chang YH, Huang YC. Type 2 diabetes increases and metformin reduces total, colorectal, liver and pancreatic cancer incidences in

Taiwanese: a representative population prospective cohort study of 800,000 individuals. *BMC Cancer* 2011; **11**: 20
75 **Li D**, Yeung SC, Hassan MM, Konopleva M, Abbruzzese JL. Antidiabetic therapies affect risk of pancreatic cancer. *Gastroenterology* 2009; **137**: 482-488
76 **Kisfalvi K**, Eibl G, Sinnett-Smith J, Rozengurt E. Metformin disrupts crosstalk between G protein-coupled receptor and insulin receptor signaling systems and inhibits pancreatic cancer growth. *Cancer Res* 2009; **69**: 6539-6545
77 **Bao B,** Wang Z, Ali S, Ahmad A, Azmi AS, Sarkar SH, Banerjee S, Kong D, Li Y, Thakur S, Sarkar FH. Metformin Inhibits Cell Proliferation, Migration and Invasion by Attenuating CSC Function Mediated by Deregulating miRNAs in Pancreatic Cancer Cells. *Cancer Prev Res* (*Phila*) 2012; Epub ahead of print
78 **Zhou Y**, Zhang E, Berggreen C, Jing X, Osmark P, Lang S, Cilio CM, Göransson O, Groop L, Renström E, Hansson O. Survival of pancreatic beta cells is partly controlled by a TCF7L2-p53-p53INP1-dependent pathway. *Hum Mol Genet* 2012; **21**: 196-207
79 **Prizment AE**, Gross M, Rasmussen-Torvik L, Peacock JM, Anderson KE. Genes related to diabetes may be associated with pancreatic cancer in a population-based case-control study in Minnesota. *Pancreas* 2012; **41**: 50-53
80 **Permert J**, Ihse I, Jorfeldt L, von Schenck H, Arnquist HJ, Larsson J. Improved glucose metabolism after subtotal pancreatectomy for pancreatic cancer. *Br J Surg* 1993; **80**: 1047-1050
81 **Yachida S**, Jones S, Bozic I, Antal T, Leary R, Fu B, Kamiyama M, Hruban RH, Eshleman JR, Nowak MA, Velculescu VE, Kinzler KW, Vogelstein B, Iacobuzio-Donahue CA. Distant metastasis occurs late during the genetic evolution of pancreatic cancer. *Nature* 2010; **467**: 1114-1117
82 **Stephens PJ**, Greenman CD, Fu B, Yang F, Bignell GR, Mudie LJ, Pleasance ED, Lau KW, Beare D, Stebbings LA, McLaren S, Lin ML, McBride DJ, Varela I, Nik-Zainal S, Leroy C, Jia M, Menzies A, Butler AP, Teague JW, Quail MA, Burton J, Swerdlow H, Carter NP, Morsberger LA, Iacobuzio-Donahue C, Follows GA, Green AR, Flanagan AM, Stratton MR, Futreal PA, Campbell PJ. Massive genomic rearrangement acquired in a single catastrophic event during cancer development. *Cell* 2011; **144**: 27-40
83 **Tanno S**, Nakano Y, Koizumi K, Sugiyama Y, Nakamura K, Sasajima J, Nishikawa T, Mizukami Y, Yanagawa N, Fujii T, Okumura T, Obara T, Kohgo Y. Pancreatic ductal adenocarcinomas in long-term follow-up patients with branch duct intraductal papillary mucinous neoplasms. *Pancreas* 2010; **39**: 36-40
84 **Vitone LJ**, Greenhalf W, McFaul CD, Ghaneh P, Neoptolemos JP. The inherited genetics of pancreatic cancer and prospects for secondary screening. *Best Pract Res Clin Gastroenterol* 2006; **20**: 253-283
85 **Canto MI**, Goggins M, Yeo CJ, Griffin C, Axilbund JE, Brune K, Ali SZ, Jagannath S, Petersen GM, Fishman EK, Piantadosi S, Giardiello FM, Hruban RH. Screening for pancreatic neoplasia in high-risk individuals: an EUS-based approach. *Clin Gastroenterol Hepatol* 2004; **2**: 606-621
86 **Huang H**, Dong X, Kang MX, Xu B, Chen Y, Zhang B, Chen J, Xie QP, Wu YL. Novel blood biomarkers of pancreatic cancer-associated diabetes mellitus identified by peripheral blood-based gene expression profiles. *Am J Gastroenterol* 2010; **105**: 1661-1669
87 **Bartosch-Härlid A**, Andersson B, Aho U, Nilsson J, Andersson R. Artificial neural networks in pancreatic disease. *Br J Surg* 2008; **95**: 817-826
88 **Honda K**, Hayashida Y, Umaki T, Okusaka T, Kosuge T, Kikuchi S, Endo M, Tsuchida A, Aoki T, Itoi T, Moriyasu F, Hirohashi S, Yamada T. Possible detection of pancreatic cancer by plasma protein profiling. *Cancer Res* 2005; **65**: 10613-10622
89 **Kelly KA**, Bardeesy N, Anbazhagan R, Gurumurthy S, Berger J, Alencar H, Depinho RA, Mahmood U, Weissleder R. Targeted nanoparticles for imaging incipient pancreatic ductal adenocarcinoma. *PLoS Med* 2008; **5**: e85
90 **Yong KT**. Biophotonics and biotechnology in pancreatic cancer: cyclic RGD-peptide-conjugated type II quantum dots for in vivo imaging. *Pancreatology* 2010; **10**: 553-564
91 **Réjiba S**, Reddy LH, Bigand C, Parmentier C, Couvreur P, Hajri A. Squalenoyl gemcitabine nanomedicine overcomes the low efficacy of gemcitabine therapy in pancreatic cancer. *Nanomedicine* 2011; **7**: 841-849
92 **Bisht S**, Mizuma M, Feldmann G, Ottenhof NA, Hong SM, Pramanik D, Chenna V, Karikari C, Sharma R, Goggins MG, Rudek MA, Ravi R, Maitra A, Maitra A. Systemic administration of polymeric nanoparticle-encapsulated curcumin (NanoCurc) blocks tumor growth and metastases in preclinical models of pancreatic cancer. *Mol Cancer Ther* 2010; **9**: 2255-2264
93 **Chenna V**, Hu C, Pramanik D, Aftab BT, Karikari C, Campbell NR, Hong SM, Zhao M, Rudek MA, Khan SR, Rudin CM, Maitra A. A polymeric nanoparticle encapsulated small-molecule inhibitor of Hedgehog signaling (NanoHHI) bypasses secondary mutational resistance to Smoothened antagonists. *Mol Cancer Ther* 2012; **11**: 165-173
94 **Glazer ES**, Zhu C, Massey KL, Thompson CS, Kaluarachchi WD, Hamir AN, Curley SA. Noninvasive radiofrequency field destruction of pancreatic adenocarcinoma xenografts treated with targeted gold nanoparticles. *Clin Cancer Res* 2010; **16**: 5712-5721
95 **Jain KK**. Advances in the field of nanooncology. *BMC Med* 2010; **8**: 83

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TOPIC HIGHLIGHT

Salvatore Gruttadauria, MD, PhD, Professor, *Series Editor*

Worldwide epidemiology of liver hydatidosis including the Mediterranean area

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Abstract

The worldwide incidence and prevalence of cystic echinococcosis have fallen dramatically over the past several decades. Nonetheless, infection with *Echinococcus granulosus* (*E. granulosus*) remains a major public health issue in several countries and regions, even in places where it was previously at low levels, as a result of a reduction of control programmes due to economic problems and lack of resources. Geographic distribution differs by country and region depending on the presence in that country of large numbers of nomadic or semi-nomadic sheep and goat flocks that represent the intermediate host of the parasite, and their close contact with the final host, the dog, which mostly provides the transmission of infection to humans. The greatest prevalence of cystic echinococcosis in human and animal hosts is found in countries of the temperate zones, including several parts of Eurasia (the Mediterranean regions, southern and central parts of Russia, central Asia, China), Australia, some parts of America (especially South America) and north and east Africa. Echinococcosis is currently considered an endemic zoonotic disease in the Mediterranean region. The most frequent strain associated with human cystic echinococcosis appears to be the common sheep strain (G1). This strain appears to be widely distributed in all continents. The purpose of this review is to examine the distribution of *E. granulosus* and the epidemiology of a re-emerging disease such as cystic echinococcosis.

Key words: Epidemiology; *Echinococcus granulosus*; Cystic echinococcosis

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INTRODUCTION

Cystic echinococcosis (CE) is a near-cosmopolitan zoonosis caused by adult or larval stages of tapeworms (cestodes) belonging to the genus *Echinococcus* (family Taeniidae). Actually, six species of *Echinococcus* have been recognized, but the most important members of the genus in respect of their public health importance and their geographical distribution are *Echinococcus granulosus* (*E. granulosus*) (which causes cystic echinococcosis) and *Echinococcus multilocularis*

(which causes alveolar echinococcosis). Infection with *E. granulosus* results in the development of one or several unilocular hydatid cysts that in humans develop mainly in the liver (70%), but also lungs (20%) and 10% of cysts can occur almost anywhere in the body (e.g., brain, body musculature, wall of the heart, kidneys, orbit of the eye, marrow cavity of bones). *E. multilocularis* metacestodes develop as a series of small, interconnected cysts, growing as a metastasising lesion almost exclusively in the liver (98%-100%), but in the later phase of infection distant metastases in other organs may occur.

E. multilocularis is a cestode whose life cycle involves a tapeworm stage during which it lives in the small intestine of carnivores (definitive hosts, usually wild or domestic canids), and a tissue-invading metacestode (larval) stage during which echinococcal cysts develop in internal organs (mainly liver and lungs) of humans and other intermediate hosts as unilocular fluid-filled bladders surrounded by a host-produced layer of granulomatous adventitial reaction. Small vesicles called brood capsules bud internally from the germinal layer and produce multiple protoscolices by asexual division. In humans, the slowly growing echinococcal cysts may reach a volume of several litres and contain many thousands of protoscolices. Moreover, internal septations and daughter cysts may appear over time, disrupting the unilocular pattern typical of the young echinococcal cysts.

Infection of an intermediate host is due to accidental ingestion of tapeworm eggs passed into the environment with faeces from definitive hosts. Transmission of *E. granulosus* could be due to domestic and wildlife reservoirs, and is influenced by human activities, behaviour, and politics.

CE represents an increasing public health and socio-economic concern in many areas of the world[1-3] and is currently considered an endemic zoonose in the Mediterranean region (MR), in addition to brucellosis, rabies, leishmaniasis and food-borne zoonotic infections[4]. Given a geographic distribution and extent greater than previously believed, several studies have shown that hydatidosis is currently considered an emerging or re-emerging disease[5,6]. The distribution and prevalence of CE depends on the presence in that country of large numbers of nomadic or semi-nomadic sheep and goat flocks that represent the intermediate host of the parasite, and their close contact with the final host, the dog, which mostly provides the transmission of infection to humans.

Molecular studies conducted on mitochondrial DNA (mtDNA) sequences, have shown that *E. granulosus* complex consists of three species and comprise ten defined strains (genotype G1-10), based on morphology, host specificity and molecular characteristics[7,8]. The intraspecific variants have substantial variation at the genetic level and DNA sequence[9], conferring several characteristics such as life-cycle patterns, host specificity, development rate, antigenicity, transmission dynamics, sensitivity to chemotherapeutic agents, and pathology[10,11]. These characteristics may have important implications for the design and development of vaccines, diagnostic reagents and drugs impacting on the epidemiology and control of echinococcosis[12,13]. Indeed, each *Echinococcus* species maintains a specific host-adapted genetic identity that only rarely overlaps in some geographical areas[5,11,14].

In this review we discuss aspects of the current epidemiology of *E. granulosus* complex and highlight worldwide and specific distribution in recognised endemic areas.

SPECIES AND DISTRIBUTION OF *E. GRANULOSUS* COMPLEX

E. granulosus has a worldwide geographical distribution with endemic foci present on every inhabited continent (Figure 1). The greatest prevalence of CE in human and animal hosts is found in countries of the temperate zones, including several parts of Eurasia (the Mediterranean regions, southern and central parts of Russia, central Asia, China), Australia, some parts of America (especially South America) and north and east Africa[2,15].

The distinct genetic types of *E. granulosus* include two sheep strains (G1 and G2), two bovid strains (G3 and G5), a horse strain (G4), a camelid strain (G6), a pig strain (G7), and a cervid strain (G8). A ninth genotype (G9) has been described in swine in Poland[8,16] and a tenth strain (G10) in reindeer in Eurasia. Among these strains, we have available data for preliminary epidemiological analyses only for some strains. In fact, some of them are still poorly characterised and further research is needed to determine with higher detail their host and geographic ranges and whether their genetic characteristics are conserved between different endemic regions.

The most frequent strain associated with human CE appears to be the common sheep strain (G1). This strain appears to be widely distributed in all continents. Highest rates of infection are recorded in communities involved in extensive sheep farming and epidemiological studies suggest that this genetic variant is the principal strain infecting humans[2,5,9,17]. Consequently, its presence coincides with areas which have high prevalence of human CE such as in Morocco, Tunisia, Kenya, Kazakhstan, western China and Argentina.

The G2 strain is known to be transmitted among sheep and infect humans also, but genetic differences biologically distinguish it from the G1 strain, conferring a different life cycle[18]. It has been found in Australia and previously also documented in Tasmania.

The G3 strain which is diffused among buffalos and transmitted by water, has been recorded in South Asia[19], but no susceptibility among humans has been found.

The G4 strain, formerly known as *Echinococcus equinus*, appears to infect exclusively equines as intermediate hosts and no human cases have been documented[9,20]. It is known to be diffused in the Mediterranean regions of Spain, Italy, Lebanon, and Syria, as well as in South Africa.

The former cattle strain (G5), known as *Echinococcus ortleppi*, is transmitted by cattle in Europe, Asia, parts of

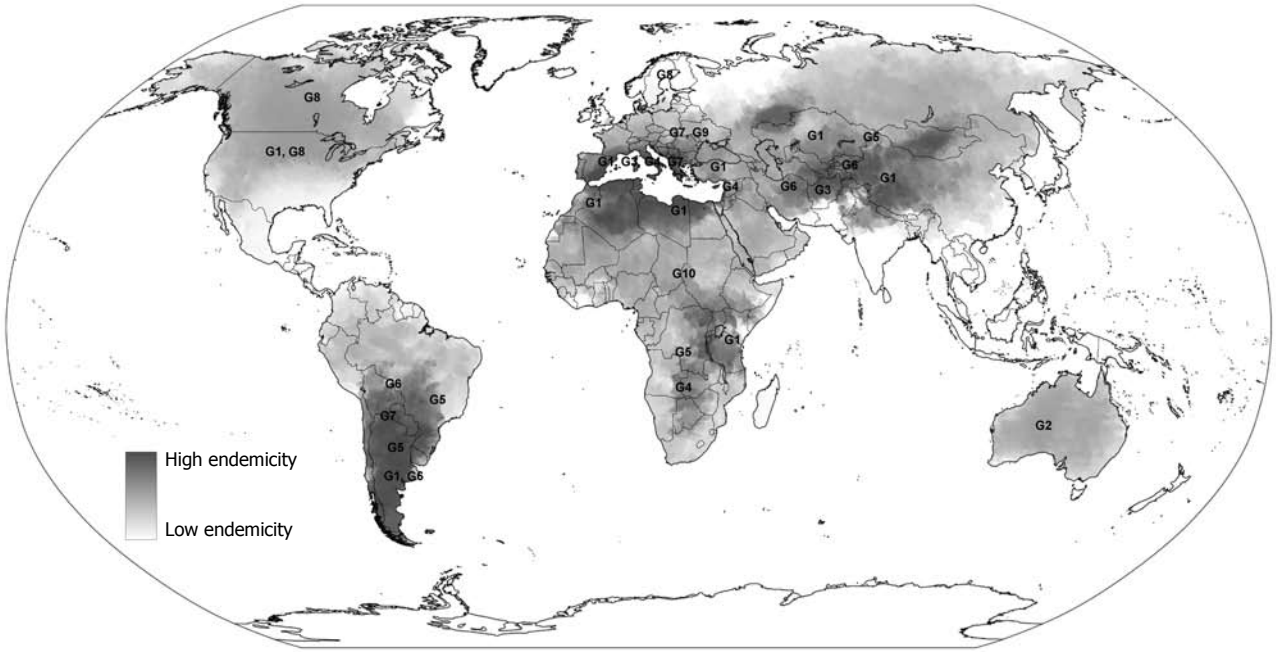

Figure 1 Worldwide distribution of the zoonotic strains of *Echinococcus granulosus* and geographical endemicity.

Africa and South America and only one case in humans has been isolated in past years[21], suggesting a less pathogenic risk for humans than the sheep strain of *E. granulosus*.

G6-10 strains are poorly distinguished from each other but they are clearly distinct from the common sheep strain[5]. The G6 strain is known to principally affect camels and goats. Animal infection is diffused in the Middle East, Africa, southern Asia and South America[9] and cases of human infection have been found in Nepal, Iran, Mauritania, Kenya and Argentina[5,17].

The G7 strain is transmitted by domestic pigs in Europe (Spain and Italy), Asia and South America, as well as the closely related genetic variant G9 that has been documented to affect Polish patients[16] although the animal reservoir is unknown.

The G8 strains are known to be transmitted between wolves and wild cervids in the northern regions of Europe, Asia and North America. Few cases of human infection have been documented with a lower severity of the disease than CE caused by other forms of *E. granulosus*[22]. However, transmission between humans of this genetic variant seems to be low and further data is needed to better assess its pathogenicity.

Finally, some other genetic variants which are poorly characterized have been found in several countries. For example, the wildlife "lion" strain transmitted among lions and wild ungulates has been documented in Africa but no human infection has been found.

EPIDEMIOLOGY OF *E. GRANULOSUS* COMPLEX: WORLDWIDE DISTRIBUTION

America

The most ubiquitous taxa of *E. granulosus* that occur in North America are the cervid strain (G8) and the sheep strain (G1). The former is diffused in wildlife mainly in Canada, Alaska and Minnesota[23]. The wildlife reservoir was found to be largely diffused among cervids and wolves, coyotes and domestic dogs[24]. EC started to be diagnosed in Canada after the 1950s following the introduction of routine chest X-rays for tuberculosis in some tribes of native Americans (such as Indians and Eskimo) who were identified with pulmonary hydatidosis[24]. In the same period, a review of 101 autochthonous cases of *E. granulosus* infection in Alaska were documented[22]. It has been estimated that 50% of moose in Ontario and British Columbia are infected with the parasite[25] and that 28%-50% of dogs in the Canadian Northwest Territories are infected with *E. granulosus*[26]. In humans, pulmonary localization is quite diffuse. Indeed, a recent chart review performed in Alberta documented 22 definite and probable cases, of which 77% were female and 41% aboriginal; 40% had pulmonary involvement and 50% hepatic involvement[27].

Sporadic autochthonous transmission among humans of the sheep strain in the western States of North America such as Arizona, California, New Mexico and Utah has been documented in reports from the 1960s[28]. The source of these *E. granulosus* infections was Australian sheep dogs imported into Utah in 1938 when the parasite diffused among sheep of this area as well as adjoining states through trading of live sheep[29]. Moreover, another source of infection were immigrants from countries in which echinococcosis disease is highly endemic, historically Icelanders, Italians and Greeks, but in more recent years, mostly persons of Middle Eastern and Asian origin. After the Second World War, foci of transmission involving swine and dogs were reported in several areas such the Mississippi valley due to the close relationship between humans and dogs[30] but transmission of infection ap-

peared to have ended by mid-century[31]. Then, an epidemic focus of sheep and human infection in western states including California, Utah, New Mexico and Arizona in the mid-1960s was traced[28]. Most infections occurred in high-risk groups such as sheep farmers and those involved in home slaughter including Basque-Americans in California[32], Mormons in central Utah[33], and Navajo and Zuni Indians in New Mexico and Arizona[33-35].

As well as in the United States, all genetic variants of the *E. granulosus* complex have been introduced into South America with domestic animals imported from other regions, such as Europe. The principal strain of *E. granulosus* is the sheep strain (G1), widely diffused in Peru, Chile, Argentina and Brazil[2].

In the central Peruvian Andes, the prevalence of hydatidosis in livestock has been noted to be 89% in sheep and 80% in cattle in a livestock raising community[36]. Among definitive hosts, the prevalence of infection in dogs in endemic areas has been reported to range from 32% to 46%[36-38] and from 46% to 88%[38-40]. The recorded surgical incidence of CE in the central and southern Peruvian Andes has been noted to be 1-2 cases per 100 000 inhabitants[36] and the prevalence of asymptomatic CE between 3% and 9.3% in rural villages in the central Peruvian highlands[38]. However, a study in a coastal city of Peru reported an annual surgical incidence of 32 per 10 000 for 1998[37] leading to the conclusion that incidence of CE is significantly under-reported. Recently, a re-emergence of transmission has been documented after the failure of previous control activities[41].

Chile is an endemic area for *E. granulosus* infection. During 2000, the prevalence of bovine, sheep and canine hydatidosis for the entire country decreased to 22.3%, 6.3% and 11%, respectively[42], after a control program[2,43,44]. With regard to human infection, although the overall incidence of diagnosed disease has been assessed as 2-2.5 cases per 100 000 inhabitants between 1992 and 2004, taking under-notification into account, the incidence has been estimated at 10 per 100 000. A major endemic area for EC is the southern part of Chile where annual surgical incidence ranged from 6 to 20 cases per 100 000 in August 2005 but reaching 162 per 100 000 in some regions[45].

In Argentina, several strains of *E. granulosus* and *E. ortleppi* have been found in different host animals and humans such as the sheep strain (G1) (mostly infecting humans), the Tasmanian sheep strain (G2), the cattle strain (G5) and the camel strain (G6)[14,46], while the pig strain (G7) has been detected in pigs and dogs but not humans[46]. The prevalence of EC affecting livestock has been documented as reaching 7% of cattle, 12.5% of sheep, 9.8% of pigs and 6.0% of goats[2]. In humans, prevalence rates depend on the endemicity of the area, ranging from 1.4 per 100 000 to 404, 260 and 30 cases per 100 000 in Neuquen, Chubut and Rio Negro (regions of Patagonia), respectively[42].

In southern Brazil only sheep strain and *E. ortleppi* have been recorded[47,48], although the most endemic area is the southern part of Brazil. Indeed, a recent analysis of hydatidosis prevalence in animals in this area reported a prevalence of infection to be 25.5% of cattle, 30.2% of sheep[42] and from 11.4% to 38% of dogs[49]. Data about human hydatidosis documented a seroprevalence of 6% in the rural population and 3.5% in the urban population of Sena Madureira[50]. However, the few data available to allow conclusions on epidemiology of different taxa often depend on control activities that are inconsistent in their consideration of the economic and public health impact of echinococcosis in these areas.

Australia

The most common strain currently found in Australia is the G1, while the G2 strain was previously also found in Tasmania[7,8,51]. This G2 strain probably evolved as a genetically modified variant after a Tasmanian hydatid control campaign aimed to strictly control helminthic diffusion among dogs. Thus, this genetic variant became dominant because of the limited gene pool on an island[18]. However, the absence of diffusion of the hydatid infection in wildlife and the intense hydatid control programmes allowed the eradication of *E. granulosus* from Tasmania in the middle 1990s[52].

In Australia several areas have been documented at high risk of transmission of *E. granulosus*, especially in wildlife. The definitive hosts most commonly involved in transmission in south eastern Australia are represented by the wild dog[53,54], while the most common intermediate hosts are grey kangaroos and wallabies[54]. Western Australia, south of Perth, is another active area of transmission of *E. granulosus*[55]. In this region, similar intermediate hosts have been found[10] while in northern Western Australia the source of infection has yet to be confirmed[56].

However, wildlife reservoirs play the main role in maintaining a constant source of transmission for domestic livestock, domestic dogs and humans[53,54,57-60]. Recent analyses assessed infection in wild dogs caught in the outer suburbs of Townsville, Queensland[61], and in those examined from the Maroochy Shire, eastern Queensland[62]. Sheep infection is still common in farms with a high number of poorly managed domestic dogs; additionally livestock are often hunted by wild dogs contaminating the pasture with eggs of *E. granulosus*[53]. However, dog and sheep infection prevalence seems to be decreasing over the last years[60], although recent surveys reported a re-emergence of domestic transmission of *E. granulosus* in some rural areas of south eastern regions where it was found that 29% of 344 rural dogs in New South Wales and 18% of 218 Victorian dogs tested positive[63].

Annually, new cases of human hydatidosis appear stable, numbering between 80 and 100 among the entire country[60,64]. Human transmission has traditionally been a public health problem of rural people due to *E. granulosus* infected domestic animals, but there is increasing potential for accidental exposure of urban residents due to the infiltration in urban centres by infected wildlife definitive hosts such as foxes and wild dogs. In fact, these animals are attracted to public recreation areas commonly fre-

quented by urban residents to scavenge food scraps[61,65]. Thus, urban residents could accidentally have direct contact with *E. granulosus* eggs through wild dog or fox faeces or *via* coprophagous flies when visiting parks and forests for recreational purposes. Furthermore, it has been documented that there has been a potential infection of the dogs of recreational pig hunters living in urban centres[66].

The reporting of hydatidosis or echinococcosis does not depend on any monitoring system but only on individual case reports. Thus, assessing accurate prevalence and incidence, as well as trend changes over time, is still difficult to achieve.

Western and Central Asia

The G1 strain, infecting sheep, goats, cattle and camels, is the most common genetic variant documented in Iran[67]. On the other hand, the G6 strain has also been found in camels, sheep and cattle in the same area[67]. Both of these were diagnosed in human hydatid infection confirming the pathogenicity of G6 for humans[67].

In Kazakstan, it has been assessed that the prevalence of infection in sheep ranges between 20%-25% in 1-year-old sheep and 74%-80% in sheep 6 years old and over. Among wild and village dogs, the prevalence of infection is 23% and 6%, respectively[68]. Although the highest worm burdens have been recorded in rural dogs, only those closer to human habitation are responsible for transmitting disease to humans[68]. Human infection has increased since the middle 1990s till present time from 200 surgical cases annually to the current level of nearly 1000 cases per year[69,70]. Similar trends in human cases have been assessed in all other Central Asian countries. However, no detailed data is available about transmission and diffusion of *E. granulosus* infection in Central Asian countries.

Hydatidosis is a serious public health problem in Turkey where *E. granulosus* infection in dogs ranges between 0.32% and 40%[71]. The predominant genotype of *E. granulosus* in Turkey is the G1 strain with a prevalence infection rate in farm animals ranging from 26.6% to 50.9% in sheep, from 13.3% to 35.68% in cattle, and reaching 22.1% in goats, 44.31% in cows and 24.39% in bulls in the most endemic areas such the Budur region[72], the Kirikkal region[73], the Afyonkarahisar district[74], and the Sivas region[75]. Lower rates in sheep (3.5%) and cattle (11.6%) have been found in less endemic areas such as Thrace region[76]. Surgical cases of human hydatidosis have been estimated to range from 0.87 to 6.6 per 100 000 inhabitants between 1987 and 1994[71]. A more recent survey based on hospital, regional and ministerial documents showed that, from 2001 to 2005, a total of 14 789 CE surgical cases were recorded with a higher incidence in the Middle Anatolian region (38.57%) and lower in the Black Sea region[77].

Several regions of the Arab peninsula such as Syria, Israel and Palestine are considered endemic for *E. granulosus*. In fact, hydatidosis is mostly associated with main risk factors such as livestock production, raising of sheep and nomadic tribal life that characterize northern Syria, northern Israel and western Palestine. Epidemiological evidence in Syria showed a prevalence of *E. granulosus* infection ranging between 9% and 15% in dogs and between 5% and 17% in livestock[78]; in Israel, ranging between 5.4% to 14.2% in dogs and between 4.56% and 10% in sheep[79,80]; and in Palestine, ranging between 7.9% and 14.3% in dogs[81]. Human infection rates have been assessed in individual studies. Annual surgical prevalence recorded from the Al-Maqased Hospital in Jerusalem was documented to be 1.76 per 100 000 inhabitants in the middle 1990s[81], while in hospitals of the Palestinian West Bank this value was 3.1 per 100 000 inhabitants, with the highest rates of 4.9, 5.0 and 5.1 per 100 000 inhabitants found in Hebron, Jericho and Bethlehem, respectively[82]. In an epidemiological study conducted in northern Israel a cumulative infection rate of 1.5 per 100 000 inhabitants was found[83], while in another study conducted in a Bedouin group from southern Israel this rate was 0.68%[83].

China

China is one of the most important endemic regions of CE[2]. The sheep strain (G1) and the camel strain (G6) are the only two *E. granulosus* strains found in China[84], both of them infectious to humans[85]. The most endemic areas for *Echinococcus spp.* have been recognized as the provinces and autonomous regions stretching from western Xinjiang[86], Ningxia and Inner Mongolia, with the highest prevalence rates occurring in pastoral communities of the eastern Tibetan plateau[87-89] (south western Qinghai and north western Sichuan) and the Tibetan autonomous area of south Gansu[90], located in western and northwestern China[85,91-95]. Infection by cysts of *E. granulosus* can be found in organs of ungulate intermediate hosts[96-99]. High prevalence of hydatid infection has been reported in sheep and yaks (99%), cattle (88%) and pigs (70%)[90]. In fact, in the western and northwestern pastoral areas of China, livestock pastoralism is a major industry with a total of 350 million sheep and other domesticated large herbivores including horses, camels, and red deer[90]. On the other hand, the definitive host is mainly represented by canids, predominantly the domestic dog. Indeed, they are kept in large populations in northwestern China for pastoralism and cultural reasons[87]. Given the close contact with local people, dogs are considered the most important definitive host transmitting *E. granulosus* to humans[2,87]. However, in certain rural regions, wild canids such as wolves and foxes are involved in the sylvatic cycle[2].

The first human CE was reported in China in 1905[86]. Over the last century, about 35 000 cases of human cystic echinococcosis have been treated surgically in China. However, given the documented 21 560 cases in Xinjiang alone with a prevalence of 80 cases/100 000 inhabitants[86], it has been assessed that an underestimation occurred in past years. Now, it has been estimated that about one million existing cases of human echinococcosis occur in China[100]. Of these, about 70% present with chronic cystic lesions of the liver as well as in other organs includ-

ing the brain[101]. The infection rate of females has been assessed to be considerably higher than that of males because of their role in the home activities including feeding dogs, collecting yak dung for fuel, and milking livestock[87,102]. Thus, nomadic or seminomadic pastoral lifestyle is one of the most important risk factors for CE in China, especially in western and northwestern areas where livestock pastoralism is a major industry[90], and women are more frequently exposed to the definitive hosts of CE. Consequently, adults have much higher infection rates than children[87], and the infection rate increases with age[102].

The increasing number of diagnosed cases may reflect improved diagnostic methods and improved outreach programs. In fact, China is now recognized as a new focus for echinococcosis research.

Africa

Although most regions of Africa are poorly researched and limited information is available, several taxa have been found in the African countries[19,103,104]. The most common strain is the G1, highly diffused in the North and East African sheep raising areas. Moreover, the exclusive presence of the camel strain (G6) has been documented. In addition, wild strains such as the *E. equinus* (the "horse strain")[105] and the "lion strain"[106] have been found in South Africa. However, the nature of *Echinococcus* in African wildlife is poorly documented.

In a recent study carried out in Libya, 25.8% of stray dogs and 21% of owned dogs have been assessed to be positive for EC[107] while another study found a prevalence of 58% of hydatidosis in the same area[108]. Nevertheless, several surveys assessed that other animals also, especially camels, are frequently infected by *E. granulosus*[109,110], while infection rates in livestock varied from 1.7% to 33.4% in sheep, 1.0% to 13.9% in cattle, 1.4% to 40.0% in camels and 0% to 18% in goats[111-113], often associated with human cases[114]. The sheep strain has been considered the most common genetic variant diffused among humans in another survey, reporting a prevalence rate of 1.7% of 20 200 patients screened by ultrasound for hydatid cysts in 36 villages along the northern coast of Libya[115] and an incidence rate of 4.2 cases per 100 000 inhabitants in Eastern Libya[112]. Indeed, in a genetic survey conducted on 179 isolates from humans collected in the border area of northwestern Kenya and south-eastern Sudan, only one was associated with the camel strain (G6) while the remaining were the common sheep strain (G1)[17]. On the other hand, other surveys conducted in central Sudan[110,116] and Egypt[117] documented the presence of human echinococcosis cases diagnosed as G6 and at least two other distinct strains (camel and equine)[118].

CE is currently of low endemicity in Egypt with a mean prevalence in dogs ranging between 3.2% in urban areas and 6% in rural areas[119]. Higher prevalence has been documented in Cairo with about 15% of dogs infected[120]. Among ruminants, confirming earlier results[121], recent data demonstrated an overall prevalence infection rate of 0.3% in sheep and goats, 0.68% in pigs, 6.4% in cows and buffaloes, 2.53% in camels[121] and 10.62% in donkeys[122]. In humans, a retrospective hospital study showed an annual surgical incidence ranging between 1.34 and 2.60 per 100 000 inhabitants[123].

In Tunisia, echinococcosis is a major public health problem due to its high prevalence and morbidity. Molecular analysis has demonstrated that the most common genetic variants of *E. granulosus* circulating in Tunisia are the G1 sheep strain and the G6 camel strain[124,125]. Sheep breeding is a significant risk factor, being practised by 94.7% of patients *vs* 58.3% of the farming population[126]. A series of studies carried out between 1999 and 2007 assessed that the prevalence of *E. granulosus* infection reached 10.41% in lambs (6-12 mo), 75.42% in sheep aged 1-2 years and 83.83 to 100% in sheep over 2 years old[127]; and 10.1% of camels[124] and 40% of sheep in a further analysis conducted in North-East Tunisia[128]. Despite the lack of recent published data, the last report of EC in humans reported an annual surgical incidence of hydatidosis of about 15 per 100 000 inhabitants[129].

In Algeria similar strain distribution has been found, identifying the sheep strain G1 infecting sheep, cattle and humans and the camel strain G6 infecting camels[130]. Dogs likely represent the main source of infection for farm animals and humans[19] with a prevalence rate of 24.8% in camels, 13.9% in cattle and 6.0% in horses[131]. Despite poor data regarding recently reported human infection, it is documented that more than 700 surgical cases are notified each year to the Ministry of Health. Last published work assessed that the annual incidence of human EC reached 3.6-4.6 per 100 000 inhabitants[132].

Morocco is considered an endemic area for echinococcosis. A genotype almost similar to the common G1 sheep strain with some nucleotide variations was found in camels and horses. Infection rate in dogs ranges from 22.0% to 62.8%, depending on the region[133]. In a more recent analysis, CE infection prevalence rates have been documented to be 10.58% in sheep, 1.88% in goats, 22.98% in cattle, 12.03% in camels and 17.80% in equines, mostly in Middle Atlas (48.72% in cattle) and in North West (37.61% in cattle and 31.65% in sheep)[134]. In humans, an annual rate of 4.55 surgical cases per 100 000 inhabitants has been documented in 2006, with a higher prevalence in the middle Atlas mountainous region[135].

Europe and the Mediterranean Basin

With the exception of Malta and the area controlled by the Government in southern Cyprus, where the disease has been practically eliminated, all the Mediterranean regions including the Arab peninsula countries are facing problems due to CE. Indeed, in Cyprus CE had an annual surgical incidence rate of 12.9 per 100 000 inhabitants before the first eradication program implemented in the 1970s and, subsequently, a second program in the 1990s[136]. In the northern part of Cyprus, disease rates

decreased from 1.95% in dogs examined in 1998-1999 to 0.012% in 2000-2003, from 23.58% to 6.61% in cattle, from 5.31% to 1.53% in sheep, while in goats rates were consistently below 0.5% and remained at 0.13%. On the other hand, the south part of Cyprus that maintained its control programme was able to keep positive testing levels at virtually 0%[137].

In Europe, *E. granulosus* is present in most countries with the exception of Ireland, Iceland and Denmark. EC of animals is rare in northern and central Europe with the exception of cervid-transmitted echinococcosis in Finland and pig-transmitted echinococcosis in regions further east. The cervid strain in Finland was found to differ genetically from the previously described North American cervid strain G8, and was identified as a new strain, G10[138]. Transmission has been documented to occur mostly between wolves, reindeer and elks[139].

The most endemic areas have been documented to be the Mediterranean regions where annual incidence rates for human CE of 4-8 per 100 000 have been reported, and parts of Eastern Mediterranean countries such as Bulgaria[2]. In some other eastern regions such as Poland, Slovakia and Ukraine, the pig strains (G6-G10) often occur as animal and sometimes human CE[140,141]. In Serbia and Montenegro the most frequent intermediate hosts for *E. granulosus* are pigs, with a percentage of infected animals ranging between 4.6% and 57.6%[142] but no information is available about human infection. Although several other countries such as Albania[78], Bosnia and Herzegovina[143,144] are recognized as endemic for CE, none of them have available published data on the exact incidence of CE in livestock, carnivores or humans.

In Greece, investigation of the prevalence and the genotype of *E. granulosus* in sheep and goats in Peloponnesus (southern Greece) revealed that sheep were infected by the G1 (sheep) strain and the G3 (buffalo) strain, while the 20 goats examined harboured the G7 (pig) strain[145]. The prevalence of CE in farm animals ranged from the mid 1980s to the mid 1990s between 82% and 56.6% in cattle, 80% and 100% in sheep, 24% and 15.4% in goats and 5% and 9.3% in pigs, while surgical human cases reached 12.9 per 100 000 inhabitants in 1984 and up to 29% in 1999[146]. Furthermore, surveillance in livestock species since 1998 has documented a prevalence of 31.3% in sheep, 10.3% in goats, 0.6% in pigs and 0% in cattle[146]. Finally, a more recent survey conducted on sheep in central Greece from 2002 to 2006, revealed an incidence rate of 39.3%[147]. In humans, the overall incidence rate was estimated to have increased from 9.77 per 100 000 in 1967[148] to 10.59 per 100 000 inhabitants in 1983[149]; results which were confirmed in another survey where an incidence of 12.7 per 100 000 inhabitants (varying from 11.6 to 13.35) has been reported[150]. Incidence rates steadily declined in the most recent survey carried out in 2007 where they have been documented to be 0.122 per 100 000 inhabitants[151]. Published data for the entire country are not available but according to personal communications with surgeons it is estimated that approximately 800 cases of cystic echinococcosis are diagnosed each year, of which between 300 and 400 of them were undergoing surgical treatment.

In Western Europe, the sheep strain (G1) is the principal cause of human CE. In the past, the cattle-based transmission cycle of *E. ortlepp* in Germany and Switzerland has been documented[2,152], but now cases are reduced to sporadic occurrence and only a single case from a human patient in the Netherlands has been reported[21].

In the United Kingdom, the parasite has a restricted distribution, being found mainly in mid and southern Wales[2,152]. Recently, a re-emergence of *E. granulosus* in Wales has been reported, noting a rise in prevalence in rural dogs between 1989 and 2002 of 3.4% to 8.1%[153].

In Spain, CE is an endemic disease in north-eastern, central and western parts of the country, with prevalence rates rising in the last few years. The most common strains found in these areas were the sheep strain (G1) infecting sheep, cattle, goats, pigs, wild boars and humans, the pig strain (G7) infecting pigs, goats and wild boars, and *E. equinus* (old G4 strain) infecting horses[154]. In the province of Alava, two recent surveys documented prevalence of *E. granulosus* infection of 8% in the dog definitive hosts[155] and 15% in Iberian wolves[156]. In the municipality of Madrid, it has been assessed that hydatidosis affected 2.88% of sheep[157]. In Laroja region, the overall prevalence has been calculated to reach 20.3% in adult sheep and up to 23% in sheep and cows in the northeastern, central and western parts of the country[158].

With regard to human hydatidosis, a higher incidence of surgical cases occurs in Salamanca, with 10.8/100 000 inhabitants affected between the end of the 1980s and 2000[159]. On the other hand, in the Laroja region, prevalence of CE decreased from 19 to 4 cases per 100 000 inhabitants until 2000[158] and in the rest of the country it ranges between 1.1 and 3.4 cases per 100 000 inhabitants[159].

In France, a surveillance system in the mid 1990s revealed a prevalence of hydatidosis of 2.5% in livestock and less than 0.28 per 100 000 in humans[160]. A higher annual incidence has been documented in Corsica (10/100 000) and eastern regions (4.5/100 000 inhabitants)[78]. In recent years, the European Centre for Disease Prevention and Control reported 17 human cases in 2005.

Italy is considered a medium to high risk country for echinococcosis. The G1 (sheep), G2 (Tasmanian sheep), G3 (buffalo), G4 (horse), and G7 (pig) genotypes of *E. granulosus* are commonly found in livestock of several regions of Italy, especially in the southern part (such as in the Campania region), in Sardinia and in Sicily[3]. Indeed, the prevalence rate of *E. granulosus* in sheep has been reported to be 5%-28% in Basilicata, 22% in Abruzzo and 47% in Tuscany[3]. In Sicily, CE was found in 67.1% of cattle, 11.13%-57.6% of sheep and 5.6%-19% of shepherd dogs[161,162]. CE prevalence of infection in Sardinia has been assessed to be 70%-92.8% of sheep, 9.4% of cattle, 9.4%-11.1% of pigs, 1% of horses and 3%-19% of dogs[3,163-166]. In Campania, the prevalence rate in cattle has been reported to range from 10.4%[167] to 14.8%[163] while in

buffalos this ranges from 10%[168] to 18.6%[169].

Infection of *E. granulosus* in animals seems to occur also in several regions of the centre of Italy while north regions could be considered of low endemicity. Indeed, in Central Italy medium prevalence values usually range from 20.2% to 47%-81.18% in sheep, from 7.34% to 15.3% in cattle, and reach 71.97% in goats, and 0.82% in pigs[170-172]. In Abruzzo, prevalence infection rates in sheep and cattle are 20.2% and 15.3%, respectively[163]. On the other hand, in Emilia Romagna the prevalences were low for several animals: 0.39%-0.54% in cattle, 0.30% in sheep, 0.39% in goats, 0.34% in horses and 0.95 per million in pigs[173]. In dogs and wolves retrieved along the whole Apennines the prevalence of *E. granulosus* infection has been noted to be 31% and 15%, respectively[172,174].

Despite these findings, the overall national occurrence of CE in farm animals can be considered low with prevalence rates of 0.52% of cattle, 1.30% of sheep, 0.6% of goats, 3.86% of sheep and goats, 0.0013% of pigs and 0.019% of horses[175]. On the other hand, human hydatidosis represents a serious public health problem, with an incidence of 1.3 cases per 100 000 inhabitants, a maximum of 4-8 cases per 100 000 inhabitants in Sardinia[176], and the occurrence of over 1000 cases requiring surgery each year[177]. Endemic zones reflect animal infection, with higher incidence rates in Sardinia and Sicily, medium in the Central-South regions, and a sporadic diffusion in the northern part of the country where this disease plays a minor role (prevalence < 1%). Annual mean incidence rates of surgical cases have been reported to be 6.6-10.6 per 100 000 inhabitants in Sardinia[178,179], 1.57-5.6 in Emilia Romagna[180,181], 1.22 in Lombardia, 2.30 in Sicily[182], 1.76 in Basilicata, 0.46 in Campania and 2.33 in Apulia[178].

Risk factors for infection are now considered to be widespread use of extensive or semi-extensive sheep farming (echinococcosis being a work-related disease), illegal slaughtering, and high numbers of sheepdogs and other types of dogs[183].

CONCLUSION

Given the wide geographic distribution, CE caused by *E. granulosus* is a re-emerging disease in several countries and regions, even in places where it was previously at low levels. Evidence suggests this is a result of a reduction of control programmes due to economic problems and lack of resources, leading to severe disease, considerable economic loss and, definitely, a public health problem of increasing concern.

REFERENCES

1 **Eckert J,** Conraths FJ, Tackmann K. Echinococcosis: an emerging or re-emerging zoonosis? *Int J Parasitol* 2000; **30**: 1283-1294

2 **Eckert J,** Schantz PM, Gasser RB, Torgerson PR, Bessonov AS, Movsessian SO, Thakur A, Grimm F, Nikogossian MA. Geographic distribution and prevalence. In: Eckert J, Gemmell MA, Meslin FX, Pawlowski Z, editors. WHO/OIE Manual on Echinococcosis in Humans and Animals: a Public Health Problem of Global Concern. Paris: Office International des Epizooties Paris, 2001: 100-142

3 **Garippa G**, Varcasia A, Scala A. Cystic echinococcosis in Italy from the 1950s to present. *Parassitologia* 2004; **46**: 387-391

4 **Sadjjadi SM**. Present situation of echinococcosis in the Middle East and Arabic North Africa. *Parasitol Int* 2006; **55** Suppl: S197-S202

5 **Thompson RC,** McManus DP. Towards a taxonomic revision of the genus Echinococcus. *Trends Parasitol* 2002; **18**: 452-457

6 **Torgerson PR,** Karaeva RR, Corkeri N, Abdyjaparov TA, Kuttubaev OT, Shaikenov BS. Human cystic echinococcosis in Kyrgystan: an epidemiological study. *Acta Trop* 2003; **85**: 51-61

7 **Pearson M,** Le TH, Zhang LH, Blair D, Dai THN, McManus DP. Molecular taxonomy and strain analysis in Echinococcus. In: Craig, P, Pawlowski, Z, editors. Cestode Zoonoses: echinococcosis and cysticercosis NATO Science Series. Series 1: life and Behavioural Sciences, vol. 341. Amsterdam: IOS Press, 2002: 205-219

8 **McManus DP**, Thompson RC. Molecular epidemiology of cystic echinococcosis. *Parasitology* 2003; **127** Suppl: S37-S51

9 **Thompson RCA,** McManus DP. Aetiology: parasites and life-cycles. In: Eckert J, Gemmell M, Meslin F-X, Pawlowski Z, editors. WHOI/OIE, Paris. Manual on echinococcosis in humans and animals: a public health problem of global concern. Geneva: World Organisation for Animal Health, 2001: 1-19

10 **Thompson RC,** Lymbery AJ. The nature, extent and significance of variation within the genus Echinococcus. *Adv Parasitol* 1988; **27**: 209-258

11 **Thompson RCA.** Biology and systematics of Echinococcus. In: Thompson RCA, Lymbery AJ, editors. Echinococcus and hydatid disease. Wallingford: CAB International, 1995: 1-50

12 **Thompson RC,** Lymbery AJ. Echinococcus: biology and strain variation. *Int J Parasitol* 1990; **20**: 457-470

13 **Bowles J,** Blair D, McManus DP. A molecular phylogeny of the genus Echinococcus. *Parasitol* 1995; **110**: 317-328

14 **Haag KL**, Ayala FJ, Kamenetzky L, Gutierrez AM, Rosenzvit M. Livestock trade history, geography, and parasite strains: the mitochondrial genetic structure of Echinococcus granulosus in Argentina. *J Parasitol* 2004; **90**: 234-239

15 **Yang YR**, Sun T, Li Z, Zhang J, Teng J, Liu X, Liu R, Zhao R, Jones MK, Wang Y, Wen H, Feng X, Zhao Q, Zhao Y, Shi D, Bartholomot B, Vuitton DA, Pleydell D, Giraudoux P, Ito A, Danson MF, Boufana B, Craig PS, Williams GM, McManus DP. Community surveys and risk factor analysis of human alveolar and cystic echinococcosis in Ningxia Hui Autonomous Region, China. *Bull World Health Organ* 2006; **84**: 714-721

16 **Scott JC**, Stefaniak J, Pawlowski ZS, McManus DP. Molecular genetic analysis of human cystic hydatid cases from Poland: identification of a new genotypic group (G9) of Echinococcus granulosus. *Parasitology* 1997; **114** (Pt 1): 37-43

17 **Dinkel A**, Njoroge EM, Zimmermann A, Wälz M, Zeyhle E, Elmahdi IE, Mackenstedt U, Romig T. A PCR system for detection of species and genotypes of the Echinococcus granulosus-complex, with reference to the epidemiological situation in eastern Africa. *Int J Parasitol* 2004; **34**: 645-653

18 **McManus DP,** Bryant C. Biochemistry, physiology and molecular biology of Echinococcus. In: Thompson RCA, Lymbery AJ, editors. Echinococcus and hydatid disease. Wallingford: CAB International 1995: 135-181

19 **Macpherson CNL,** Wachira TWM. Cystic echinococcosis in Africa south of the Sahara. In: Andersen FL, Ouhelli H, Kachani M, editors. Compendium of Cystic Echinococcosis in Africa and in Middle Eastern Countries with special Ref-

erence to Morocco. Provo: Brigham Young University, 1997: 245-277
20 **Thompson RC,** Lymbery AJ, Constantine CC. Variation in Echinococcus: towards a taxonomic revision of the genus. *Adv Parasitol* 1995; **35**: 145-176
21 **Bowles J,** van KF, McManus D. Cattle strain of Echinococcus granulosus and human infection. *Lancet* 1992; **339**: 1358
22 **Wilson JF**, Diddams AC, Rausch RL. Cystic hydatid disease in Alaska. A review of 101 autochthonous cases of Echinococcus granulosus infection. *Am Rev Respir Dis* 1968; **98**: 1-15
23 **Peterson RO.** Wolf Ecology and Prey Relationships on Isle Royale. Washington DC: United States Printing Office, 1997
24 **Webster GA**, Cameron TW. Epidemiology and diagnosis of echinococcosis in Canada. *Can Med Assoc J* 1967; **96**: 600-607
25 **Lamy AL,** Cameron BH, LeBlanc JG, Culham JA, Blair GK, Taylor GP. Giant hydatid lung cysts in the Canadian northwest: outcome of conservative treatment in three children. *J Pediatr Surg* 1993; **28**: 1140-1143
26 **Moore RD**, Urschel JD, Fraser RE, Nakai SS, Geeraert AJ. Cystic hydatid lung disease in northwest Canada. *Can J Surg* 1994; **37**: 20-22
27 **Somily A**, Robinson JL, Miedzinski LJ, Bhargava R, Marrie TJ. Echinococcal disease in Alberta, Canada: more than a calcified opacity. *BMC Infect Dis* 2005; **5**: 34
28 **Schantz PM,** Chai J, Craig PS, Eckert J, Jenkins DJ, Macpherson CNL, Thakur A. Epidemiology and Control of hydatid disease. In: Thompson RCA, Lymbery AJ, editors. Echinococcus and Hydatid Disease. Wallingford: CAB International, 1995: 231-233
29 **Crellin JR**, Andersen FL, Schantz PM, Condie SJ. Possible factors influencing distribution and prevalence of Echinococcus granulosus in Utah. *Am J Epidemiol* 1982; **116**: 463-474
30 **Pappaioanou M**, Schwabe CW, Sard DM. An evolving pattern of human hydatid disease transmission in the United States. *Am J Trop Med Hyg* 1977; **26**: 732-742
31 **Hutchison WF**. Studies on the hydatid worm, Echinococcus granulosus. II. Prevalence in Missippi. *Am J Trop Med Hyg* 1960; **9**: 612-615
32 **Schantz PM**, Clérou RP, Liu IK, Schwabe CW. Hydatid disease in the central valley of California. Transmission of infection among dogs, sheep, and man in Kern county. *Am J Trop Med Hyg* 1970; **19**: 823-830
33 **Andersen FL.** Introduction to cystic echinococcosis and description of cooperative research project in Morocco. In: Andersen FL, Ouhelli H, Kachani M, editors. Compendium on Cystic Echinococcosis. Brigham: Young Univ Press; 1997: 1-17
34 **Schantz PM**, von Reyn CF, Welty T, Schultz MG. Echinococcosis in Arizona and New Mexico. Survey of hospital records, 1969-1974. *Am J Trop Med Hyg* 1976; **25**: 312-317
35 **Schantz PM**, Alstine CV, Blacksheep A, Sinclair S. Prevalence of Echinococcus granulosus and other cestodes in dogs on the Navajo reservation in Arizona and New Mexico. *Am J Vet Res* 1977; **38**: 669-670
36 **Moro PL**, McDonald J, Gilman RH, Silva B, Verastegui M, Malqui V, Lescano G, Falcon N, Montes G, Bazalar H. Epidemiology of Echinococcus granulosus infection in the central Peruvian Andes. *Bull World Health Organ* 1997; **75**: 553-561
37 **Moro PL**, Lopera L, Cabrera M, Cabrera G, Silva B, Gilman RH, Moro MH. Short report: endemic focus of cystic echinococcosis in a coastal city of Peru. *Am J Trop Med Hyg* 2004; **71**: 327-329
38 **Moro PL**, Gilman RH, Verastegui M, Bern C, Silva B, Bonilla JJ. Human hydatidosis in the central Andes of Peru: evolution of the disease over 3 years. *Clin Infect Dis* 1999; **29**: 807-812
39 **Moro PL,** Bonifacio N, Gilman RH, Lopera L, Silva B, Takumoto R, Verastegui M, Cabrera L. Field diagnosis of Echinococcus granulosus infection among intermediate and definitive hosts in an endemic focus of human cystic echinococcosis. *Trans R Soc Trop Med Hyg* 1999; **93**: 611-615
40 **Lopera L**, Moro PL, Chavez A, Montes G, Gonzales A, Gilman RH. Field evaluation of a coproantigen enzyme-linked immunosorbent assay for diagnosis of canine echinococcosis in a rural Andean village in Peru. *Vet Parasitol* 2003; **117**: 37-42
41 **Dueger EL,** Gilman RH. Prevalence, intensity, and fertility of ovine cystic echinococcosis in the central Peruvian Andes. *Trans R Soc Trop Med Hyg* 2001; **95**: 379-383
42 Panamerican Health Organization. Report of the Southern Cone subregional project on echinococcosis control and surveillance Argentina, Brasil, Chile and Uruguay: first meeting; 2004 July 7-9; Montevideo, Uruguay. Montevideo: OPS, 2004
43 **Alvarez F,** Tamayo R, Ernst S. Estimación de la prevalencia de echinococcosis canina en la XII Región, Chile, 2002. *Parasitol Latinoam* 2005; **60**: 74-77
44 **Serra I**, Araneda J, Araya C, Serra V. [Regional analysis of human and animal hydatidosis in Chile, 1989-1993]. *Bol Chil Parasitol* 1996; **51**: 3-12
45 **Apt W,** Perez C, Galdamez E, Campano S, Vega F, Vargas D, Rodriguez J, Retamal C, Cortes P, Zulantay I, de Rycke PH. Echinococcosis/hydatidosis in the VII Region of Chile: diagnosis and educational intervention. *Rev Panam Salud Publica* 2000; **7**: 8-16
46 **Kamenetzky L,** Gutierrez AM, Canova SG, Haag KL, Guarnera EA, Parra A, Garcia GE, Rosenzvit MC. Several strains of Echinococcus granulosus infect livestock and humans in Argentina. *Infect Genet Evol* 2002; **2**: 129-136
47 **Haag KL**, Araújo AM, Gottstein B, Siles-Lucas M, Thompson RC, Zaha A. Breeding systems in Echinococcus granulosus (Cestoda; Taeniidae): selfing or outcrossing? *Parasitology* 1999; **118** (Pt 1): 63-71
48 **De la Rue ML,** Silva KLMV, Dinkel A, Mackenstedt U, Romig T. New data on Echinococcus pp.in southern Brazil. *Int Arch Hydatidosis* 2004; **35**: 61
49 **Farias LN,** Malgor R, Cassaravilla C, Braganca C, de la Rue ML. Echinococcosis in southern Brazil: efforts toward implementation of a control program in Santana do Livramento, Rio Grande do Sul. *Rev Inst Med Trop Sao Paulo* 2004; **46**: 153-156
50 **Pastore R**, Vitali LH, Macedo Vde O, Prata A. [A serological survey of the infection by Echinococcus sp. in the municipality of Sena Madureira, AC]. *Rev Soc Bras Med Trop* 2003; **36**: 473-477
51 **Jenkins DJ**. Hydatid control in Australia: where it began, what we have achieved and where to from here. *Int J Parasitol* 2005; **35**: 733-740
52 **Beard TC,** Bramble AJ, Middleton MJ. Eradication in Our Lifetime: A Log Book of the Tasmanian Hydatid Control Programs, 1962-1996. Hobart: Department of Primary Industries, Water & Environment, 2001
53 **Grainger HJ,** Jenkins DJ. Transmission of hydatid disease to sheep from wild dogs in Victoria, Australia. *Int J Parasitol* 1996; **26**: 1263-1270
54 **Jenkins DJ**, Morris B. Echinococcus granulosus in wildlife in and around the Kosciuszko National Park, south-eastern Australia. *Aust Vet J* 2003; **81**: 81-85
55 **Jenkins DJ**, Macpherson CN. Transmission ecology of Echinococcus in wild-life in Australia and Africa. *Parasitology* 2003; **127** Suppl: S63-S72
56 **Lymbery AJ**, Thompson RC, Kruger JG. The geographic distribution of hydatid infection in cattle in Western Australia. *Aust Vet J* 1995; **72**: 430-432
57 **Hope M**, Bowles J, Prociv P, McManus DP. A genetic comparison of human and wildlife isolates of Echinococcus granulosus in Queensland: public health implications. *Med J Aust* 1992; **156**: 27-30
58 **Jenkins DJ**, Morris B. Unusually heavy infections of Echinococcus granulosus in wild dogs in south-eastern Australia.

Aust Vet J 1991; **68**: 36-37
59 **Thompson RC**, Nott DB, Squire J, Rennell D. Evidence that the Australian sylvatic strain of Echinococcus granulosus is infective to humans. *Med J Aust* 1987; **146**: 396-397
60 **Jenkins DJ**, Power K. Human hydatidosis in New South Wales and the Australian Capital Territory, 1987-1992. *Med J Aust* 1996; **164**: 18-21
61 **Brown B**, Copeman DB. Zoonotic importance of parasites in wild dogs caught in the vicinity of Townsville. *Aust Vet J* 2003; **81**: 700-702
62 **Jenkins DJ**, Allen L, Goullet M. Encroachment of Echinococcus granulosus into urban areas in eastern Queensland, Australia. *Aust Vet J* 2008; **86**: 294-300
63 **Jenkins DJ**, McKinlay A, Duolong HE, Bradshaw H, Craig PS. Detection of Echinococcus granulosus coproantigens in faeces from naturally infected rural domestic dogs in south eastern Australia. *Aust Vet J* 2006; **84**: 12-16
64 **Jenkins DJ.** Echinococcus in Australia. In: Torgerson P, Shaikenov B, editors. Echinococcosis in Central Asia: problems and solutions. Almaty, Duir: INTAS Network Project 01-0505, 2004: 125-130
65 **Jenkins DJ**, Craig NA. The role of foxes Vulpes vulpes in the epidemiology of Echinococcus granulosus in urban environments. *Med J Aust* 1992; **157**: 754-756
66 **Thompson RC**, Lymbery AJ, Hobbs RP, Elliot AD. Hydatid disease in urban areas of Western Australia: an unusual cycle involving western grey kangaroos (Macropus fuliginosus), feral pigs and domestic dogs. *Aust Vet J* 1988; **65**: 188-190
67 **Harandi MF**, Hobbs RP, Adams PJ, Mobedi I, Morgan-Ryan UM, Thompson RC. Molecular and morphological characterization of Echinococcus granulosus of human and animal origin in Iran. *Parasitology* 2002; **125**: 367-373
68 **Shaikenov BSh,** Torgerson P. Changes in the epidemiology of echinococcosis in Kazakhstan. In: Torgerson P, Shaikenov B, editors. Echinococcosis in central Asia: problems and solutions. Almaty, Duir: INTAS Network Project 01-0505, 2004: 3-12
69 **Torgerson PR,** Shaikenov BS, Kuttubaev O. Cystic echinococcosis in Central Asia: New epidemic in Kazakhstan and Kyrgystan. In: Craig P, Pawlowski Z, editors. Cestode Zoonoses: Echinococcosis and cysticercosis. Oxford: IOS Press, 2000: 99-105
70 **Torgerson PR,** Shaikenov BS, Baitursinov KK, Abdybekova AM. The emerging epidemic of echinococcosis in Kazakhstan. *Trans R Soc Trop Med Hyg* 2002; **96**: 124-128
71 **Altintas N.** Past to present: echinococcosis in Turkey. *Acta Trop* 2003; **85**: 105-112
72 **Umur S**. Prevalence and economic importance of cystic echinococcosis in slaughtered ruminants in Burdur, Turkey. *J Vet Med B Infect Dis Vet Public Health* 2003; **50**: 247-252
73 **Yildiz K**, Tunçer C. [Prevalence of hydatid cysts in cattle in the province of Kirikkale.]. *Turkiye Parazitol Derg* 2005; **29**: 247-250
74 **Köse M**, Sevimli FK. Prevalence of cystic echinococcosis in slaughtered cattle in Afyonkarahisar. *Turkiye Parazitol Derg* 2008; **32**: 27-30
75 **Aciöz M**, Celiksöz A, Ozçelik S, Değerli S. [Prevalence of cyst hydatic in slaughtered cattle between April and May 2005 in Sivas]. *Turkiye Parazitol Derg* 2008; **32**: 205-207
76 **Esatgil MU**, Tüzer E. Prevalence of hydatidosis in slaughtered animals in Thrace, Turkey. *Turkiye Parazitol Derg* 2007; **31**: 41-45
77 **Utuk AE**, Simsek S, Koroglu E, McManus DP. Molecular genetic characterization of different isolates of Echinococcus granulosus in east and southeast regions of Turkey. *Acta Trop* 2008; **107**: 192-194
78 **Seimenis A,** Battelli G. Epidemiological situation and surveillance. Information Circular-WHO Mediterrean Zoonoses Control Centre, Issue dedicated on cystic echinococcosis in the Mediterranean countries, 2003; 6-8
79 **Shimshony A**. Epidemiology of emerging zoonoses in Israel. *Emerg Infect Dis* 1997; **3**: 229-238
80 **Furth M.** Echinococcosis in Northern Israel: prevalence in domestic and wild animals. International Symposium on Environmental Adaptation of Echinococcus; 1998 Aug; Hokaido, Japan. Japan: Hokaido, 1998: 18-20
81 **Abdel-Hafez SK,** Kamhawi S. Cystic echinococcosis in the Levant countries (Jordan, Palestinian Authority, Israel, Syria, and Lebanon). In: Anderson FL, Ouheli H, Kachani M, editors. Compendium on Cystic Echinococcosis in Africa and Middle Eastern Countries with Special Reference to Morocco. Pravo: Brigham Young University, 1997: 292-316
82 **Abu-Hasan N,** Daragmeh M, Adwan K, Al-Qaoud K, Abdel-Hafez SK. Human cystic echinococcosis in the West Bank of Palestine: surgical incidence and seroepidemiological study. *Parasitol Res* 2002; **88**: 107-112
83 **Youngster I,** Hoida G, Craig PS, Sneir R, El-On J. Prevalence of cystic echinococcosis among Muslim and Jewish populations in southern Israel. *Acta Trop* 2002; **82**: 369-375
84 **Zhang YL,** Bart JM, Wen H, Ma XD, Miao YQ, Lin RY. Molecular evidence of sheep (G1) and camel (G6) strains of Echinococcus granulosus in Xinjiang, China. *Zhongguo Ji Sheng Chong Bing Fang Zhi Zazhi* 2005; **18**: 333-335
85 **Bart JM**, Abdukader M, Zhang YL, Lin RY, Wang YH, Nakao M, Ito A, Craig PS, Piarroux R, Vuitton DA, Wen H. Genotyping of human cystic echinococcosis in Xinjiang, PR China. *Parasitology* 2006; **133**: 571-579
86 **Chi P**, Zhang W, Zhang Z, Hasyet M, Liu F, Ding Z, Andersen FL, Tolley HD, Schantz PM. Cystic echinococcosis in the Xinjiang/Uygur Autonomous Region, People's Republic of China. I. Demographic and epidemiologic data. *Trop Med Parasitol* 1990; **41**: 157-162
87 **Tiaoying L**, Jiamin Q, Wen Y, Craig PS, Xingwang C, Ning X, Ito A, Giraudoux P, Wulamu M, Wen Y, Schantz PM. Echinococcosis in Tibetan populations, western Sichuan Province, China. *Emerg Infect Dis* 2005; **11**: 1866-1873
88 **Qiu JM,** Liu F, Schantz PM, Ito A, Delker C, He JG. Epidemiological survey of hydatidosis in Tibetan areas of western Sichuan province. *Archivos Internacionales de la Hidatidosis* 1999; **33**: 84
89 **Chai JJ**. Epidemiological studies on cystic echinococcosis in China--a review. *Biomed Environ Sci* 1995; **8**: 122-136
90 **Craig PS.** Epidemiology of echinococcosis in Western China. In: Torgerson P, Shaikenov B, editors. Echinococcosis in central Asia: problems and solutions. Almaty, Dauir: INTAS Network Project 01-0505, 2004: 43-58
91 **Giraudoux P,** Craig PS, Delattre P, Bao G, Bartholomot B, Harraga S, Quere JP, Raoul F, Wang Y, Shi D, Vuitton DA. Interactions between landscape changes and host communities can regulate Echinococcus multilocularis transmission. *Parasitol* 2003; **127** Suppl: S121-S131
92 **Giraudoux P**, Pleydell D, Raoul F, Quéré JP, Wang Q, Yang Y, Vuitton DA, Qiu J, Yang W, Craig PS. Transmission ecology of Echinococcus multilocularis: what are the ranges of parasite stability among various host communities in China? *Parasitol Int* 2006; **55** Suppl: S237-S246
93 **Xiao N**, Qiu J, Nakao M, Li T, Yang W, Chen X, Schantz PM, Craig PS, Ito A. Echinococcus shiquicus n. sp., a taeniid cestode from Tibetan fox and plateau pika in China. *Int J Parasitol* 2005; **35**: 693-701
94 **Yang YR**, Rosenzvit MC, Zhang LH, Zhang JZ, McManus DP. Molecular study of Echinococcus in west-central China. *Parasitology* 2005; **131**: 547-555
95 **Tang CT**, Wang YH, Peng WF, Tang L, Chen D. Alveolar Echinococcus species from Vulpes corsac in Hulunbeier, Inner Mongolia, China, and differential development of the metacestodes in experimental rodents. *J Parasitol* 2006; **92**: 719-724
96 **Musinov M.** The epizootiology of echinococcosis in animals in Uzbekistan. *Med Parazitol* (*Mosk*) 1998; 40-41

97 **Oksanen A,** Laaksonen S. Faecal survey of dogs for echinococcosis and other internal parasites in north-eastern Finland. *Suomen Elainlaakarilehti* 1995; **101**: 171-172

98 **Kilani M,** Lancia A, Jemli MH, Darghouth MA, Jomaa I, Diouani F. Prevalence of Echinococcus granulosus in captured dogs in Tunisia (parasitic necropsy). *Archivos Internacionales de la Hidatidosis* 1997; **32**: 276

99 **Kimura H,** Furuya K, Kawase S, Sato C, Takahashi K, Uraguchi K. Epidemiology of alveolar echinococcosis in Hokkaido, Japan. *Archivos Internacionales de la Hidatidosis* 1999; **33**: 85-89

100 **Ito A,** Romig T, Takahashi K. Perspective on control options for Echinococcus multilocularis with particular reference to Japan. *Parasitol* 2003; **127** Suppl: S159-S172

101 **Wen H,** Yang WG. Public health importance of cystic echinococcosis in China. *Acta Trop* 1997; **67**: 133-145

102 **Wang Q**, Qiu J, Yang W, Schantz PM, Raoul F, Craig PS, Giraudoux P, Vuitton DA. Socioeconomic and behavior risk factors of human alveolar echinococcosis in Tibetan communities in Sichuan, People's Republic of China. *Am J Trop Med Hyg* 2006; **74**: 856-862

103 **Ibrahem MM,** Gusbi AM. Cystic echinococcosis in North Africa (excluding Morocco): veterinary aspects. In: Andersen FL, Ouhelli H, Kachani M, editors. Compendium of Cystic Echinococcosis in Africa and in Middle Eastern Countries with special Reference to Morocco. Provo: Brigham Young University, 1997: 207-222

104 **Kachani M,** Ouhelli H, Kadiri A, El Hasnaoui M. Prevalence of hydatid cysts in livestock in Morocco and potential role of these intermediate hosts in transmission of cystic echinococcosis. In: Andersen FL, Ouhelli H, Kachani M, editors. Compendium of Cystic Echinococcosis in Africa and in Middle Eastern Countries with special Reference to Morocco. Provo: Brigham Young University, 1997: 156-168

105 **Kumaratilake LM,** Thompson RC, Eckert J. Echinococcus granulosus of equine origin from different countries possess uniform morphological characteristics. *Int J Parasitol* 1986; **16**: 529-540

106 **Verstr AJ**. Review of Echinococcus species in South Africa. *Onderstepoort J Vet Res* 1965; **32**: 7-118

107 **Buishi IE**, Njoroge EM, Bouamra O, Craig PS. Canine echinococcosis in northwest Libya: assessment of coproantigen ELISA, and a survey of infection with analysis of risk-factors. *Vet Parasitol* 2005; **130**: 223-232

108 **Ben Musa NA**, Sadek GS. Prevalence of echinococcosis in street dogs in Tripole District, Libya. *J Egypt Soc Parasitol* 2007; **37**: 793-800

109 **Kassem HH,** Gdoura NK. Hydatidosis in camels (Camelus dromedarius) slaughtered at Sirt Abattoir, Libya. *J Egypt Soc Parasitol* 2006; **36**: 1-10

110 **Elmahdi IE**, Ali QM, Magzoub MM, Ibrahim AM, Saad MB, Romig T. Cystic echinococcosis of livestock and humans in central Sudan. *Ann Trop Med Parasitol* 2004; **98**: 473-479

111 **Al-Khalidi NW.** Cystic echinococcosis (hydatidosis) in sheep, goats, cattle and camels in Shahat Abattoir, Al-Jabal, Libya. In: Proceedings of the Third Annual Meeting for Animal Production Under Arid Conditions, United Arab Emirates University, vol. 1, 1998: 143-149

112 **Tashani OA**, Zhang LH, Boufana B, Jegi A, McManus DP. Epidemiology and strain characteristics of Echinococcus granulosus in the Benghazi area of eastern Libya. *Ann Trop Med Parasitol* 2002; **96**: 369-381

113 **Ibrahem MM,** Crellin JR. Prevalence of cystic echinococcosis in camels (Camelus dromedarius) in Libya. *J Helminthol* 1998; **72**: 27-31

114 **Bardonnet K,** Piarroux R, Schneegans F, Dia L, Beurdeley A, Vuitton DA. Hydatidosis in Mauritania: a camel strain infectious to humans. *Int Arch Hydatidosis* 2001; **34**: 20

115 **Shambesh MA**, Craig PS, Macpherson CN, Rogan MT, Gusbi AM, Echtuish EF. An extensive ultrasound and serologic study to investigate the prevalence of human cystic echinococcosis in northern Libya. *Am J Trop Med Hyg* 1999; **60**: 462-468

116 **Omer RA,** Dinkel A, Romig T, Mackenstedt U, Elamin M, Elnahas A, Aradaib I. Strains characterization of human hydatidosis in Sudan. *Int Arch Hydatidosis* 2004; **35**: 41

117 **Azab ME**, Bishara SA, Helmy H, Oteifa NM, El-Hoseiny LM, Ramzy RM, Ahmed MA. Molecular characterization of Egyptian human and anima Echinococcus granulosus isolates by RAPD-PCR technique. *J Egypt Soc Parasitol* 2004; **34**: 83-96

118 **Derbala AA.** Current status of hydatidosis/echinococcosis: guidelines for surveillance, siology, diagnosis and recommendations for prevention and control in Egypt. 29th World Congress of the World Small Animal Vet. Ass.; 2004 Oct 06-09; Rhodes, Greece

119 **El Shazly AM**, Awad SE, Nagaty IM, Morsy TA. Echinococcosis in dogs in urban and rural areas in Dakahlia Governorate, Egypt. *J Egypt Soc Parasitol* 2007; **37**: 483-492

120 **Mazyad SA**, Mahmoud LH, Hegazy MM. Echinococcosis granulosus in stray dogs and Echino-IHAT in the hunters in Cairo, Egypt. *J Egypt Soc Parasitol* 2007; **37**: 523-532

121 **Haridy FM**, Ibrahim BB, Elshazly AM, Awad SE, Sultan DM, El-Sherbini GT, Morsy TA. Hydatidosis granulosus in Egyptian slaughtered animals in the years 2000-2005. *J Egypt Soc Parasitol* 2006; **36**: 1087-1100

122 **Haridy FM**, Abdel Gawad AG, Ibrahim BB, Hassan AA, El-Sherbi GT, El Shazly AM, Morsy TA. Zoonotic hydatidosis in donkeys: post mortum examination in the Zoo, Giza, Egypt. *J Egypt Soc Parasitol* 2008; **38**: 305-312

123 **Kandeel A**, Ahmed ES, Helmy H, El Setouhy M, Craig PS, Ramzy RM. A retrospective hospital study of human cystic echinococcosis in Egypt. *East Mediterr Health J* 2004; **10**: 349-357

124 **Lahmar S**, Debbek H, Zhang LH, McManus DP, Souissi A, Chelly S, Torgerson PR. Transmission dynamics of the Echinococcus granulosus sheep-dog strain (G1 genotype) in camels in Tunisia. *Vet Parasitol* 2004; **121**: 151-156

125 **M'rad S**, Filisetti D, Oudni M, Mekki M, Belguith M, Nouri A, Sayadi T, Lahmar S, Candolfi E, Azaiez R, Mezhoud H, Babba H. Molecular evidence of ovine (G1) and camel (G6) strains of Echinococcus granulosus in Tunisia and putative role of cattle in human contamination. *Vet Parasitol* 2005; **129**: 267-272

126 **Benabid M,** Chahed MK, Nouira R, Galai Y, Bourabtine A, Aoun K. Knowledge, behaviour and implications on hydatidosis in Tunisia. *Rev Tun Infectiol* 2007; **4**: 22-428

127 **Lahmar S**, Kilani M, Torgerson PR, Gemmell MA. Echinococcus granulosus larvae in the livers of sheep in Tunisia: the effects of host age. *Ann Trop Med Parasitol* 1999; **93**: 75-81

128 **Lahmar S**, Chéhida FB, Pétavy AF, Hammou A, Lahmar J, Ghannay A, Gharbi HA, Sarciron ME. Ultrasonographic screening for cystic echinococcosis in sheep in Tunisia. *Vet Parasitol* 2007; **143**: 42-49

129 **Anon T.** The surgical incidence rate of hydatidosis in Tunisia (1988-1992). Report of the D.S.S.B. (Direction de Santé et des Soins de base). Tunis: Ministry Public Health, 1993

130 **Bart JM**, Bardonnet K, Elfegoun MC, Dumon H, Dia L, Vuitton DA, Piarroux R. Echinococcus granulosus strain typing in North Africa: comparison of eight nuclear and mitochondrial DNA fragments. *Parasitology* 2004; **128**: 229-234

131 **Bardonnet K,** Benchikh-Elfegoun MC, Bart JM, Harraga S, Hannache N, Haddad S, Dumon H, Vuitton DA, Piarroux R. Cystic echinococcosis in Algeria: cattle act as reservoirs of a sheep strain and may contribute to human contamination. *Vet Parasitol* 2003; **116**: 35-44

132 **Shambesh MK.** Human cystic echinococcosis in North Africa (excluding Morocco). In: Anderson FL, Ouhelli H, Kachani M, editors. Compendium on Cystic Echinococcosis in Africa and in Middle Eastern Countries with Special Ref-

erence to Morocco. Provo: Brigham Young University, 1997: 223-244
133 **Ouhelli H,** Kadiri A, El Hasnaoui M, Kachani M. Prevalence of Echinococcus granulosus in dogs in Morocco and potential role of dogs in transmission of cystic echinococcosis. In: Anderson FL, Ouhelli H, Kachani M, editors. Compendium on Cystic Echinococcosis in Africa and in Middle Eastern Countries with Special Reference to Morocco. Provo: Brigham Young University, 1997: 145-155
134 **Azlaf R**, Dakkak A. Epidemiological study of the cystic echinococcosis in Morocco. *Vet Parasitol* 2006; **137**: 83-93
135 **Kachani M,** Macpherson CN, Lyagoubi M, Berrada M, Bouslikhane M, Kachani F, El HM. Public health education/importance and experience from the field. Educational impact of community-based ultrasound screening surveys. *Acta Trop* 2003; **85**: 263-269
136 **Economides P**, Christofi G. Evaluation of control programmes for echinococcosis/hydatidosis in Cyprus. *Rev Sci Tech* 2000; **19**: 784-792
137 **Christofi G,** Deplazes P, Christofi N, Tanner I, Economides P, Eckert J. Screening of dogs for Echinococcus granulosus coproantigen in a low endemic situation in Cyprus. *Vet Parasitol* 2002; **104**: 299-306
138 **Lavikainen A**, Lehtinen MJ, Meri T, Hirvelä-Koski V, Meri S. Molecular genetic characterization of the Fennoscandian cervid strain, a new genotypic group (G10) of Echinococcus granulosus. *Parasitology* 2003; **127**: 207-215
139 **Hirvela-Koski V,** Haukisalmi V, Kilpela SS, Nylund M, Koski P. Echinococcus granulosus in Finland. *Vet Parasitol* 2003; **111**: 175-192
140 **Kedra AH,** TKach VV, Swiderski Z, Pawlowski Z, Emets A, Pawlowski J. Molecular characterisation of Echinococcus granulosus from a wild boar. *Acta Parasitol* 2000; **45**: 121-122
141 **Snábel V**, D'Amelio S, Mathiopoulos K, Turceková L, Dubinský P. Molecular evidence for the presence of a G7 genotype of Echinococcus granulosus in Slovakia. *J Helminthol* 2000; **74**: 177-181
142 **Ivanovic S,** Pavlovic I. Conference Technologija mesa (Yugoslavia). *Meat Technol* 1999; **40**: 302-303
143 **Obradović Z**, Zerem E, Beslagić Z, Susić A. [Echinococcosis in Bosnia and Herzegovina]. *Med Arh* 2006; **60**: 259-262
144 **Guska S,** Cerimagic Z, Pilav I. Conservative surgical treatment of pulmonary hydatid disease in children. *Med Arh* 2007; **61**: 11-15
145 **Varcasia A,** Tosciri G, Pedes T, Pipia AP, Marrosu R, Scala A, Garippa G. Cystic echinococcosis in pigs and wald boars of Sardinia (Italy). Proc 6th International Symposium on the Mediterranean Pig; 2007 Oct 11-13; Messina-Capo d'Orlando, Italy
146 **Sotiraki S,** Himonas C, Korkoliakou P. Hydatidosis-echinococcosis in Greece. *Acta Trop* 2003; **85**: 197-201
147 **Christodoulopoulos G**, Theodoropoulos G, Petrakos G. Epidemiological survey of cestode-larva disease in Greek sheep flocks. *Vet Parasitol* 2008; **153**: 368-373
148 **Karpathios T**, Fretzayas A, Nicolaidou P, Papadellis F, Vassalos M, Tselentis J, Thomaidis T, Matsaniotis N. Statistical aspects of hydatid disease in Greek adults. *Am J Trop Med Hyg* 1985; **34**: 124-128
149 **Matsaniotis N**, Karpathios T, Koutoyzis J, Nicolaidou P, Fretzayas A, Papadellis F, Thomaidis T. Hydatid disease in Greek children. *Am J Trop Med Hyg* 1983; **32**: 1075-1078
150 **Papadopoulos G.** Echinococcosis/hydatidosis in the world. Epizootiological and epidemiological analysis: problems in the Mediterranean area. Abstr. XIII Congreso International de Hidatologia. Madrid, 1985: 21-24
151 Office International des Epizooties. World Animal Health Information Data Base. Office International des Epizooties, 2007. Available from: URL: http//www.oie.int//wahis/public.php
152 **Eckert J**, Thompson RC. Echinococcus strains in Europe: a review. *Trop Med Parasitol* 1988; **39**: 1-8
153 **Buishi I,** Walters T, Guildea Z, Craig P, Palmer S. Reemergence of canine Echinococcus granulosus infection, Wales. Emerg Infect Dis serial online, 2005-04; 11(4). Available from: URL: http//www.cdc.gov/ncidod/EID/vol11no4-0178.htm
154 **Daniel Mwambete K**, Ponce-Gordo F, Cuesta-Bandera C. Genetic identification and host range of the Spanish strains of Echinococcus granulosus. *Acta Trop* 2004; **91**: 87-93
155 **Benito A**, Carmena D, Joseph L, Martínez J, Guisantes JA. Dog echinococcosis in northern Spain: comparison of coproantigen and serum antibody assays with coprological exam. *Vet Parasitol* 2006; **142**: 102-111
156 **Sobrino R**, Gonzalez LM, Vicente J, Fernández de Luco D, Garate T, Gortázar C. Echinococcus granulosus (Cestoda, Taeniidae) in the Iberian wolf. *Parasitol Res* 2006; **99**: 753-756
157 **Rodrigo MR,** Canizares FJ, Nieto GA, Lopez FCR, Alcalde MS. Ovine Hydatidosis in the Community of Madrid (Spain). *Rev Panam Salud Publica* 1997; **5**: 376-379
158 **Jimenez S,** Perez A, Gil H, Schantz P, Ramalle E, Juste R. Progress in control of cystic echinococcosis in La Rioja, Spain: decline in infection prevalences in human and animal hosts and economic costs and benefits. *Acta Trop* 2002; **83**: 213-221
159 **Pardo J,** Muro A, Galindo I, Cordero M, Carpio A, Siles-Lucas M. [Hydatidosis in the province of Salamanca (Spain): should we let down our guard?]. *Enferm Infecc Microbiol Clin* 2005; **23**: 266-269
160 **Bichet H**, Dorchies P. [Estimation of the prevalence of bovine hydatid cyst in the south Pyrenees]. *Parasite* 1998; **5**: 61-68
161 **Poglayen G,** Brianti E, Russo A, Gaglio G, Sorgi C, Giannetto S. Old dreams, new vision: Cystic echinococosis in Sicily. *WAAVP* 2003; **19**: 164
162 **Giannetto S**, Poglayen G, Brianti E, Sorgi C, Gaglio G, Canu S, Virga A. An epidemiological updating on cystic echinococcosis in cattle and sheep in Sicily, Italy. *Parassitologia* 2004; **46**: 423-424
163 **Garippa G**. Updates on cystic echinococcosis (CE) in Italy. *Parassitologia* 2006; **48**: 57-59
164 **Varcasia A**, Canu S, Lightowlers MW, Scala A, Garippa G. Molecular characterization of Echinococcus granulosus strains in Sardinia. *Parasitol Res* 2006; **98**: 273-277
165 **Scala A**, Garippa G, Varcasia A, Tranquillo VM, Genchi C. Cystic echinococcosis in slaughtered sheep in Sardinia (Italy). *Vet Parasitol* 2006; **135**: 33-38
166 **Varcasia A**, Garippa G, Scala A. The diagnosis of Echinococcus granulosus in dogs. *Parassitologia* 2004; **46**: 409-412
167 **Rinaldi L,** Maurelli MP, Capuano F, Perugini AG, Veneziano V, Cringoli S. Molecular update on cystic echinococcosis in cattle and water buffaloes of southern Italy. *Zoonoses Public Health* 2008; **55**: 119-123
168 **Capuano F,** Rinaldi L, Maurelli MP, Perugini AG, Veneziano V, Garippa G, Genchi C, Musella V, Cringoli G. Cystic echinococcosis in water buffaloes: epidemiological survey and molecular evidence of ovine (G1) and buffalo (G3) strains. *Vet Parasitol* 2006; **137**: 262-268
169 **Cringoli G**, Rinaldi L, Musella V, Veneziano V, Maurelli MP, Di Pietro F, Frisiello M, Di Pietro S. Geo-referencing livestock farms as tool for studying cystic echinococcosis epidemiology in cattle and water buffaloes from southern Italy. *Geospat Health* 2007; **2**: 105-111
170 **Bio C,** Fagiolo A. Incidence of Hydatidosis in sheep slaughterhouse in the province of Mazzo, Italy. *Parassitologia* 2004; **46**: 28
171 **Piergili Fioretti D**, Diaferia M, Veronesi F, Sammarone E. Distribution of hydatidosis in slaughtered animals in Umbria region from 1995 to 2004: a retrospective analysis. *Parassitologia* 2004; **46**: 437-438
172 **Giangaspero A,** Paoletti B, Gatti A, Iorio R, Traversa D, Capelli G, Manfredi MT, Varcasia A, Garippa G. The epide-

miological scenario on echinococcosis in the Abruzzo region. *Parassitologia* 2006; **48**: 338
173 **Faggioli P,** Baldelli R, Battelli G. Cystic echinococcosis in Italy: prevalence in food-producing animals slaughtered in the Emilia-Romagna Region. 6th National Conference of Parasitology of the Bulgarian Society for Parasitology; 2001 Oct 5-7; Sofia, Bulgaria. Bulgaria: Sofia, 2001: 121
174 **Guberti V**, Bolognini M, Lanfranchi P, Battelli G. Echinococcus granulosus in the wolf in Italy. *Parassitologia* 2004; **46**: 425-427
175 **EFSA.** The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union in 2006. *EFSA J* 2006; **130**: 207-216
176 **Pozio E**. Epidemiology and control prospects of foodborne parasitic zoonoses in the European Union. *Parassitologia* 2008; **50**: 17-24
177 **Dionigi G**, Carrafiello G, Recaldini C, Sessa F, Boni L, Rovera F, Dionigi R. Laparoscopic resection of a primary hydatid cyst of the adrenal gland: a case report. *J Med Case Reports* 2007; **1**: 61
178 **Gabriele F**, Bortoletti G, Conchedda M, Palmas C, Ecca AR. [Human cystic hydatidosis in Italy: a public health emergency? Past to present]. *Parassitologia* 2004; **46**: 39-43
179 **Conchedda M,** Antonelli A, Caddori A, Seu V, Gabriele F. Human Cystic Echinococcosis in Sardinia from 2001 to 2005 based on Hospital discharge Reports. *Parassitologia* 2008; **50**: 40
180 **Ostanello F**, Piccolomini LL, Martinello F, Vizioli M, Battelli G. [Echinococcosis/hydatidosis in Emilia-Romagna: a study of hospital admissions in the period of 1989-1993]. *Epidemiol Prev* 1997; **21**: 41-47
181 **Battelli G**, Ostanello F, Baldelli R, Di Francesco A, Grilli R, Vizioli M. Human echinococcosis in the Emilia-Romagna region (northern Italy) in the years 1997 to 2002: an updating. *Parassitologia* 2004; **46**: 415-416
182 **Silva KT**. Malaria eradication as a legacy of colonial discourse: the case of Sri Lanka. *Parassitologia* 1994; **36**: 149-163
183 **Kern P**, Bardonnet K, Renner E, Auer H, Pawlowski Z, Ammann RW, Vuitton DA, Kern P. European echinococcosis registry: human alveolar echinococcosis, Europe, 1982-2000. *Emerg Infect Dis* 2003; **9**: 343-349

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TOPIC HIGHLIGHT

Salvatore Gruttadauria, MD, PhD, Professor, *Series Editor*

Multidisciplinary imaging of liver hydatidosis

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Abstract

Liver hydatidosis is a parasitic endemic disease affecting extensive areas in our planet, a significant stigma within medicine to manage because of its incidence, possible complications, and diagnostic involvements. The diagnosis of liver hydatidosis should be as fast as possible because of the relevant complications that may arise with disease progression, involving multiple organs and neighboring structures causing disruption, migration, contamination. The aim of this essay is to illustrate the role of imaging as ultrasonography (US), multi detector row computed tomography, and magnetic resonance imaging (MRI) in the evaluation of liver hydatidosis: the diagnosis, the assessment of extension, the identification of possible complications and the monitoring the response to therapy. US is the screening method of choice. Computed tomography (CT) is indicated in cases in which US is inadequate and has high sensitivity and specificity for calcified hydatid cysts. Magnetic resonance is the best imaging procedure to demonstrate a cystic component and to show a biliary tree involvement. Diagnostic tests such as CT and MRI are mandatory in liver hydatidosis because they allow thorough knowledge regarding lesion size, location, and relations to intrahepatic vascular and biliary structures, providing useful information for effective treatment and decrease in post-operative morbidity. Hydatid disease is classified into four types on the basis of their radiologic appearance.

Key words: Liver hydatidosis; Hepatic cyst; Daughter cysts; Calcified cyst; Pericyst

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INTRODUCTION

Hydatid disease is a worldwide zoonosis caused by the larval stage of the echinococcus tapeworm, that is endemic in many parts of the world (in European, Middle Eastern, Mediterranean, South American and African countries)[1-4]. There are two types of Echinococcus infections: Echinococcus granulosis, the more common type, and Echinococcus multilocularis, the less common but more invasive. Hydatid disease is a relevant health problem in underdeveloped areas where veterinary control does not exist. The

most frequent location of hydatid cystic lesions is in the liver (up to 80% of cases), followed by the lung (about 20% of cases), and with a lower reported incidence in any other organ or tissue in the body[1-4].

Dogs or other carnivores are definitive hosts, whereas sheep or other ruminants are intermediate hosts. Humans are secondarily infected by the ingestion of food or water contaminated by dog feces containing the eggs of the parasite. After the ingestion of the eggs, the freed embryo enters a branch of the portal vein by passing through the duodenal mucosa; most of these embryos become lodged in the hepatic capillaries where they either die or grow into hydatid cysts. Some embryos pass through the hepatic capillaries and become lodged in the lungs and other organs.

The definitive diagnosis of liver echinococcosis requires a combination of imaging, serologic, and immunologic studies[4].

At biochemical analysis, there is usually eosinophilia, and a serologic test is positive in 25% of patients[5]. At histopathologic analysis, a hydatid cyst is composed of three layers: the outer pericyst, which corresponds to compressed liver tissue; the endocyst, an inner germinal layer; and the ectocyst, a translucent thin interleaved membrane[5].

Imaging procedures are essential in diagnosis and evaluation of the extent of liver hydatidosis; ultrasound (US), computed tomography (CT), and magnetic resonance (MR) can depict hydathid disease[2-4,6].

The imaging method used depends on the involved organ, and the radiologic findings range from purely cystic lesions to a completely solid appearance[3]. US is the screening method of choice and is also used to monitor efficacy of medical therapy[2-4,6]. CT is always performed because it has a high sensitivity (94%)[7]. It is an important preoperative diagnostic tool to determine vascular, biliary or extrahepatic extension, to recognize complications, such as rupture and infections, and therefore to assess respectability[8-10]. MR is the best imaging procedure to demonstrate a cystic component. It helps to determine vascular or biliary tree involvement, as well as extrahepatic extension[10,11].

There are many potential complications such as exophytic growth, transdiaphragmatic thoracic involvement, peritoneal seeding, biliary communication, portal vein involvement, abdominal wall invasion and hematogenous dissemination in any anatomic location (lung, kidney, spleen, bone, brain)[6,10,11].

RADIOLOGIC FINDINGS

Ultrasonographic findings

The ultrasonographic appearance of hydatid cysts may vary, from a simple aspect to a more complex one, in relation to the stage of evolution and maturity[5-7,10]. US can categorize cysts as solitary univesicular, solitary multivesicular, solid echogenic mass, multiple, either uni- or multivesicular, or collapsed, flattened and calcified[8].

In the first stage, the hydatid cyst may manifest as a well-defined anechoic cyst (Figure 1), an anechoic cyst except for hydatid "sand"[2,6,7]. The more complex aspect is typical of the advanced stages and is related to the presence of multiple internal septa, daughter cysts, multiple echogenic foci and floating membranes inside the cavity (Figures 2 and 3)[7-9]. Membranes may appear as serpentine linear structures, a finding that is highly specific for hydatid disease[7,12,13]. The detachment of the membrane inside the cyst is considered the US "water lily sign"[13-16]. The cyst wall is visible as double echogenic lines separated by a hypoechogenic layer (Figure 3)[16].

Multivesicular cysts manifest as well-defined fluid collections in a honeycomb pattern with multiple septa representing the wall of the daughter cysts. Daughter cysts

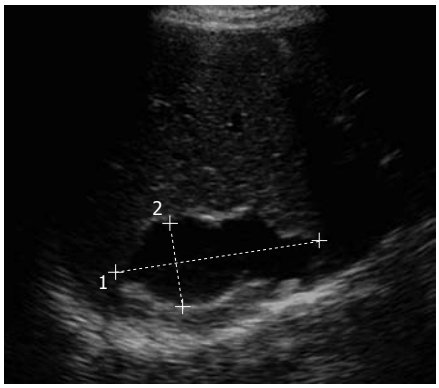

Figure 1 Liver hydatid disease in a 50-year-old man appears as a well-defined anechoic mass without hydatid sand and septa (type I).

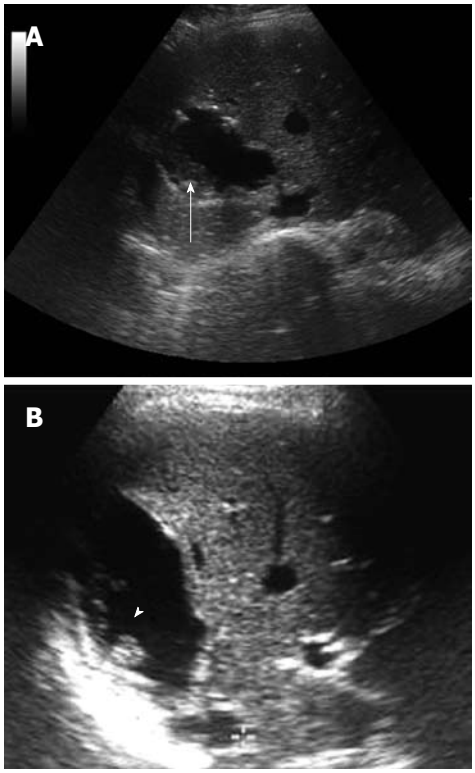

Figure 2 Liver hydatidosis in a 27-year-old female. Ultrasonography images (A, B) show a lesion with mixed echogenicity, with hydatid sand (the arrow) and multiple echogenic foci (the arrowhead).

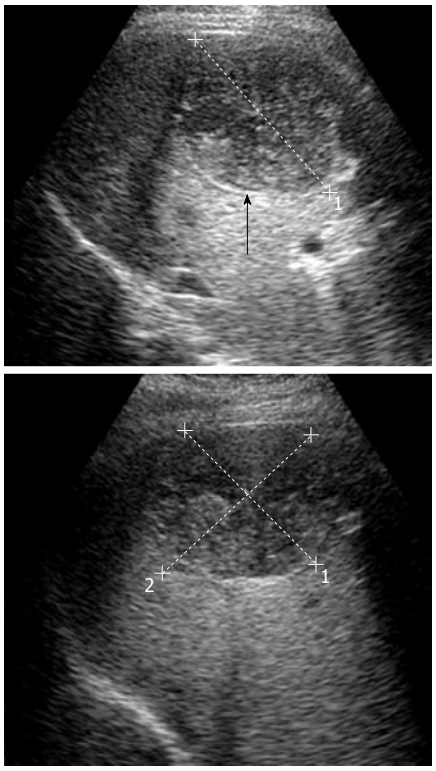

Figure 3 Ultrasonography images of hydatid disease show multiple internal septa and floating membranes inside the cyst. Note the cyst wall is visible as double echogenic lines (see the black arrow).

appear as cysts within a cyst[7-9]. Altering patient's position may change the position of daughter cysts.

The more complex aspects of hydatid cyst may also mimic solid hepatic masses, and differential diagnosis becomes difficult but fundamental; it is important to look for daughter vesicles or membranes within the lesion that may help in differential diagnosis[7,8]. Cyst calcification is seen in dead hydatid cysts; US shows a hyperechogenic contour with a cone-shaped acoustic shadow[13,15,16].

When the cyst wall is heavily calcified, only the anterior portion of the wall is visualized and appears as a thick arch with a posterior concavity. Partial calcification of the cyst does not indicate the death of the parasite, on the contrary densely calcified cysts may be assumed to be inactive[8,9].

US is considered the preferred investigatory test to monitor efficacy of medical antihydatid therapy because of its low cost[12,14]. Positive response findings include reduction in cyst size, membrane detachment, progressive increase in cyst echogenicity and mural calcification[12].

Computed tomography findings

CT is indicated in cases in which US is inadequate due to patient-related difficulties (obesity, excessive intestinal gas, previous surgery[3,5-7]. CT has high sensitivity and specificity for hepatic hydatid disease[7]. Intravenous administration of contrast medium is useful to give a vascular map to the surgeon, and when complications (especially infection and communication with the biliary tree) and extrahepatic diffusion are suspected.

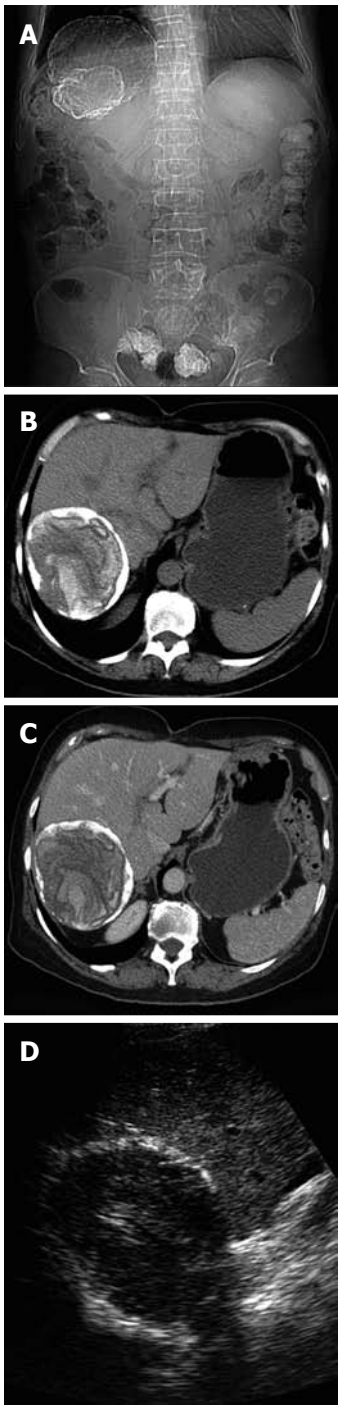

Figure 4 Calcified unilocular hydatid cyst. Digital scout image (A) shows a round, densely calcified lesion supra-elevating the right diaphragm. Computed tomography basal (B) and contrast-enhanced (C) images reveal a hypoattenuating lesion with peripheral wall calcification in the right lobe. Membranes appear as serpentine linear structures. Note the complex ultrasonography aspect of the cyst and the hyperechoic wall (D).

CT may show the same findings as US[6,7]. Calcification of the cyst wall, internal septa, floating membranes and daughter vesicles are easily detected at CT[3,5].

A hydatid cyst typically is seen as a round lesion with water attenuation density, surrounded by a calcified ring-like (Figure 4) or highly attenuated wall, representing the pericyst (Figure 5)[17]. Detachment of the laminated mem-

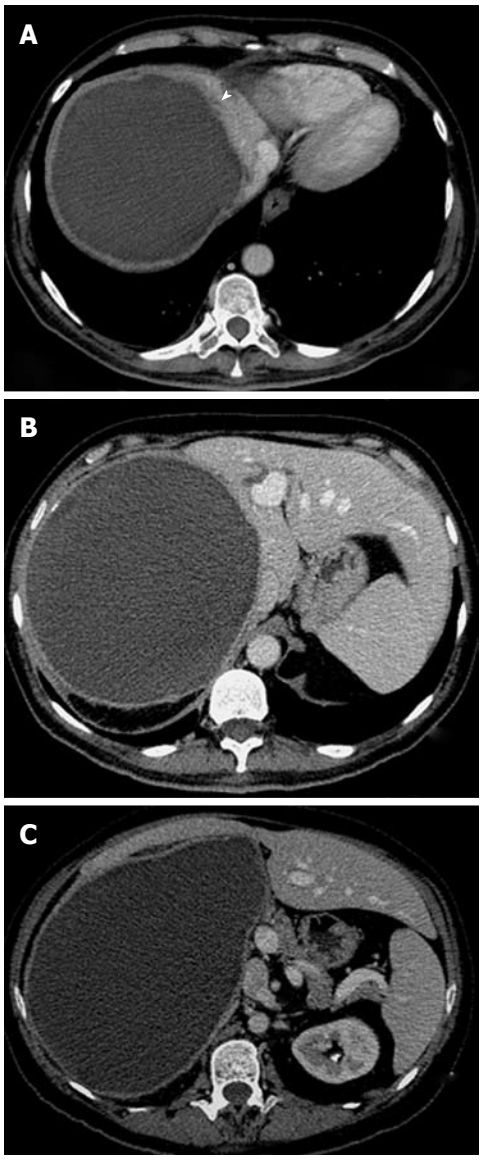

Figure 5 Computed tomography scan shows a huge nonenhancing mass with regular contours and thickened wall representing the pericyst (the white arrowhead) occupying all the right lobe of the liver (type Ⅰ); either the right portal vein or the right hepatic vein is completely replaced.

branes from the pericyst are visualized as linear areas of increased attenuation within the cyst[17].

At CT daughter vesicles are visible as round structures located peripherally within the mother cyst; they usually contain fluid with a lower attenuation than that of the fluid of the mother cyst (Figure 6)[5,7].

Contrast-enhanced CT may show the typical high-attenuation rim representing abscesses surrounding the lesion. Sometimes, patchy areas of contras-enhanced liver parenchyma are seen in the vicinity of the lesion, representing inflammatory changes[18].

The dead cysts are totally calcified and at CT they appear as round hyperattenuating areas (Figure 7)[5,7].

CT also may depict gas or air-fluid levels or fat inside the hydatid cyst, indirect signs of infection and/or communication with the biliary tree (Figures 8 and 9)[6,10,11].

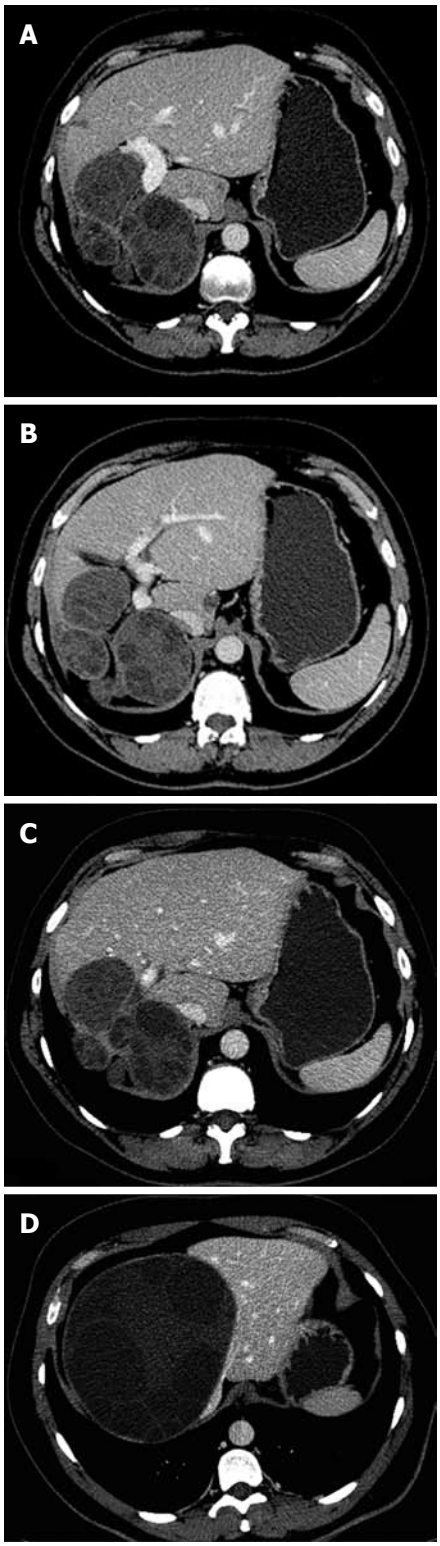

Figure 6 Computed tomography scan (A, B, C, D) shows some unenhanced hypoattuenuating masses with well defined borders occupying the right lobe of the liver; multiple round daughter cysts are seen peripherally inside the lesion (type Ⅱ). Note the "rosette appearance".

CT is the modality of choice to study extra-hepatic diffusion because it allows imaging of the entire abdomen, pelvis and thorax.

Extra-hepatic diffusion may regard peritoneum, the

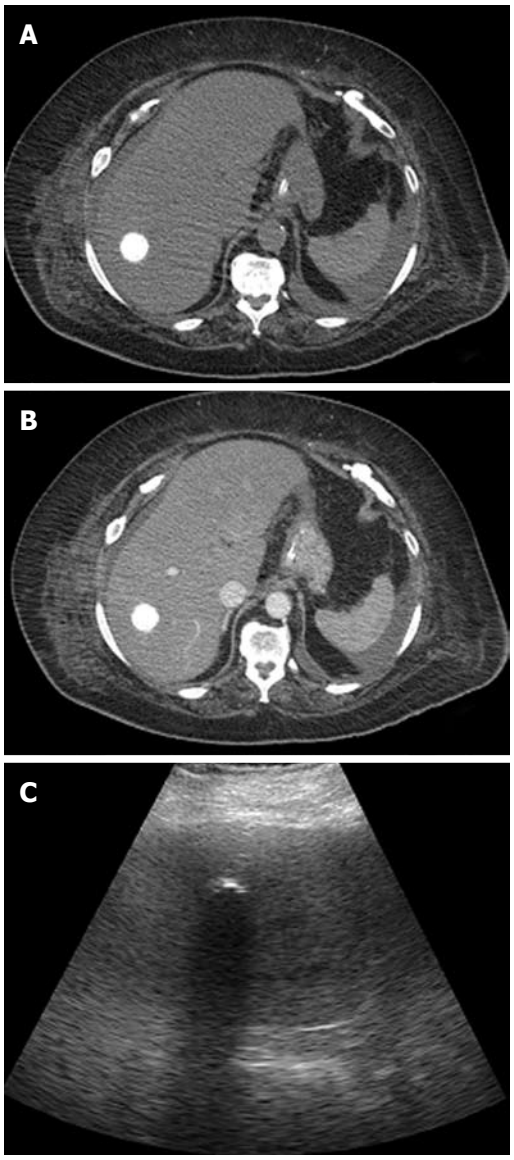

Figure 7 Seventy-three year-old-woman with a dead hydatid cyst. Computed tomography scan basal and enhanced images (A, B) show a totally calcified cyst (type Ⅲ). At ultrasonography (C) calcified cyst shows strong posterior shadowing.

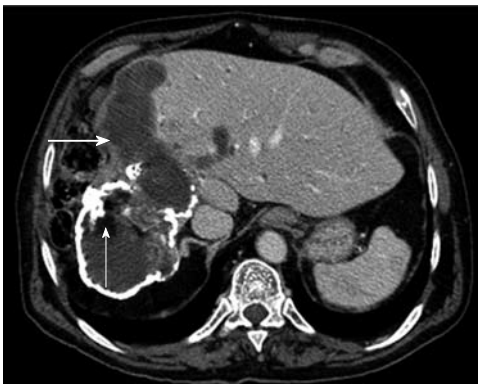

Figure 8 Contrast-enhanced upper abdominal computed tomography scan demonstrates a partially calcified hydatid cyst in direct communication with the biliary tree markedly dilated (the bold white arrow). The presence of fat components within the cyst derives from the lipid elements in bile (the thin white arrow) (type Ⅳ).

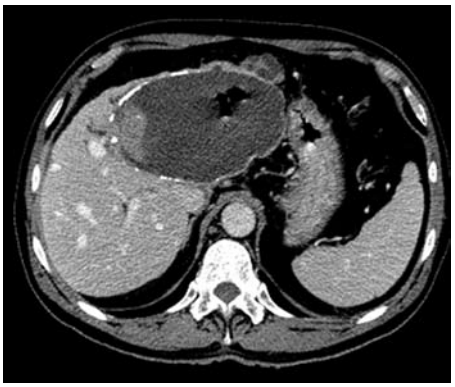

Figure 9 Fifty-five-year-old male with acute abdominal pain, fever and leukocytosis. Contrast-enhanced computed tomography shows intracystic gas in the anterior part of a unilocular partially calcified hydatid cyst, suggesting super-infection; finding confirmed at surgery (type Ⅳ).

diaphragm and the thorax cavity, the abdominal wall, the portal system, and the hematogenous dissemination[4,6,7].

Magnetic resonance findings

MR may be performed to confirm the hypothesis of hepatic hydatidosis and visualize the lesion in different planes. It is the best diagnostic investigation to differentiate the cystic component from the others and to demonstrate a biliary tree involvement[13].

The hydatid cysts may show variable signal intensities on T1- and T2-weighted images, according to the different components inside the lesion[13,17,18].

The necrotic and the fluid components are hypointense on T1-weighted images and markedly hyperintense on T2-weighted images[13,18,19].

When present the daughter cysts are seen as cystic structures attached to the germinal layer that are hypointense relative to the intracystic fluid on T1-weighted images (Figure 10)[13].

The characteristic sign of hydatid disease is represented by the pericyst that usually appears as a low-signal-intensity rim on T2-weighted images (Figure 10)[17,19].

In addition, there may be an intermediate-signal-intensity inner ring representing the detachment of the membranes[19].

After the i.v. injection of gadolinium contrast agent the pericyst may show slight enhancement (Figure 11).

MR is the best diagnostic tool in demonstrating the floating membranes (Figure 12) and irregularities of the rim representing incipient detachment of the membranes (Figure 13)[17,19,20]. On the other hand MR is less sensitive than CT scan in showing cyst wall calcification.

The "snake sign" is another typical MR imaging feature: it represents collapsed parasitic membranes, secondary to damage or degeneration of the hydatid cyst: these membranes have low signal intensity with all sequences (Figure 13).

Intracystic air-fluid level may be visible on MR, as a possible sign of super-infection (Figure 14)[16,21].

MR cholangiopancreatography (MRCP) is useful to

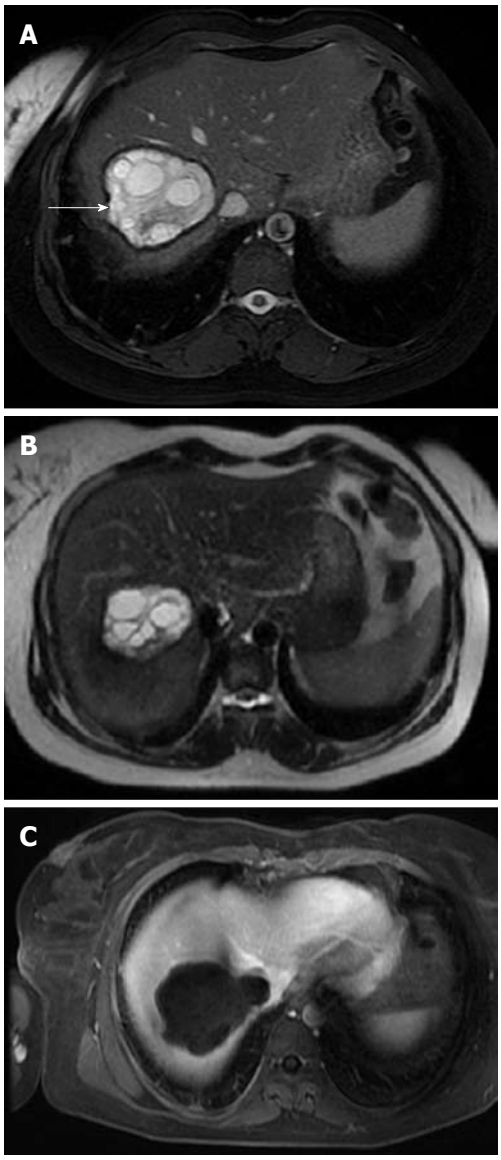

Figure 10 Axial T1-weighted (A) and T2-weighted (B) images show a well defined cystic lesion and the typical peripheral location of the daughter cysts within the mother cyst. Note the marked hypointensity of the pericyst (the white arrow). After contrast administration (C) the cystic lesion does not show contrast enhancement (type Ⅱ).

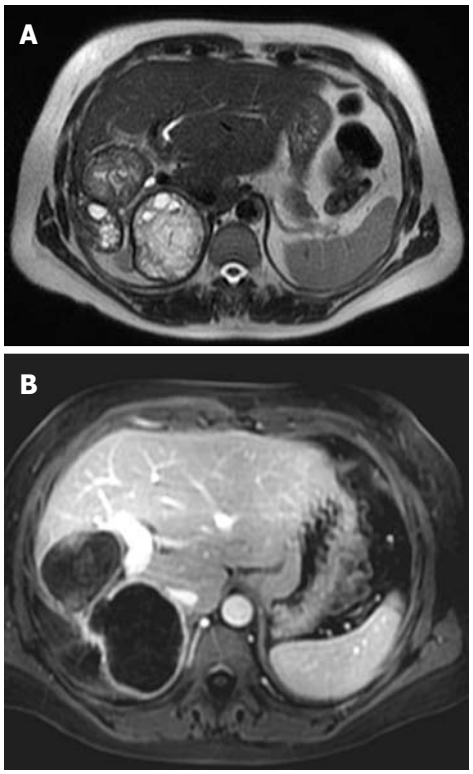

Figure 11 Axial T2-weighted and post-contrast images show the exophytic growth of hydatid cyst (type Ⅱ) (A). After the injection of contrast media the septa and cyst wall enhance (B). Note the proximity of the cyst to the diaphragm which facilitates transdiaphragmatic thoracic involvement and to main portal vein.

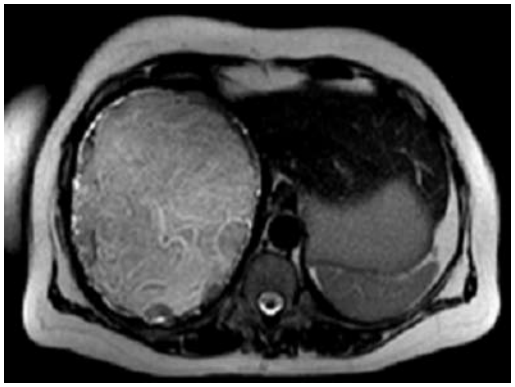

Figure 12 35-year-old woman living in an endemic region. Axial T2-weighted magnetic resonance image show the hydatid cyst that occupies almost the entire right lobe of the liver with thickened pericyst and multiple floating membranes inside the lesion (type Ⅱ).

study potential involvement of the biliary tree: communication between the cysts and the biliary tree; dilatation of the biliary system secondary to compression of the hydatid cyst[20,21] (Figure 15).

It is known that routine MRI does not adequately differentiate completely liquid hydatid cysts (type Ⅰ, see following paragraph) from simple cysts: Inan *et al*[22,23] have demonstrated in their study that diffusion-weighted (DW-MRI), a recent MRI technique, can be helpful in the differential diagnosis.

DW-MRI has long been used exclusively in brain imaging due to technical problems and sensitivity to motion artifacts (caused by cardiac motion and respiration); with the advent of faster sequences, DW-MRI has been applied to abdominal imaging[22,23].

Using DW MRI with a high b factor (1000 s/mm^2) the hydatid cysts are hyperintense, whereas none of the simple cysts show significant hyperintensity (Figure 16)[22-24].

In addition, using DW MRI it is possible to calculate a parameter, called the apparent diffusion coefficients (ADCs), that measures the difference in cellular density of hepatic lesions[22-24].

This quantitative parameter can be used to differentiate hydatid cysts from simple cysts. The difference between the ADCs of the hydatid cysts and those of simple cysts can be attributed to the difference in cyst contents[22-24].

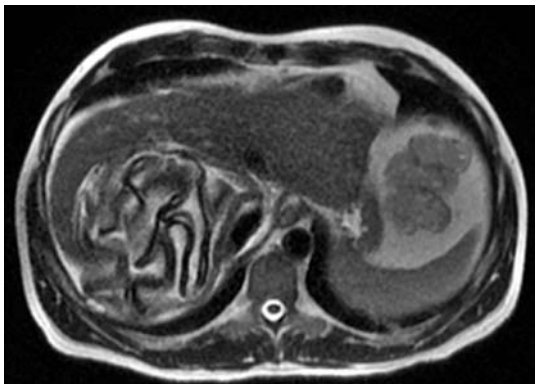

Figure 13 The detachment of the pericyst and the collapsed membranes inside the cyst due to damage or degeneration may give the hydatid cyst a serpentine linear aspect; this is the "snake sign".

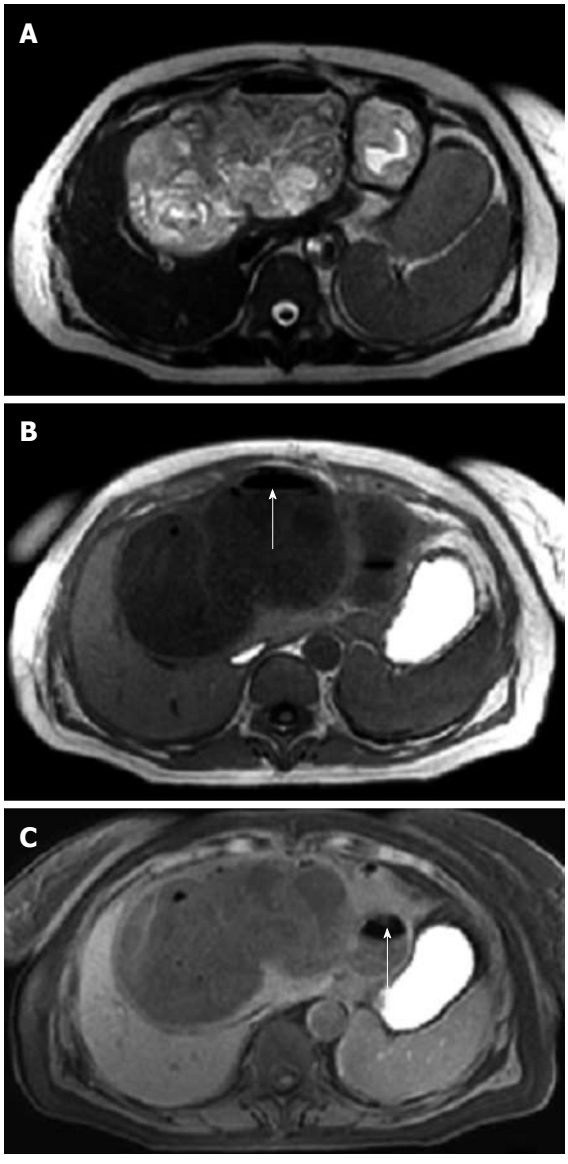

Figure 14 Axial T2-weighted (A) and T1-weighted (B, C) magnetic resonance images demonstrate a round, cystic lesion in the left hepatic lobe, with thickened pericyst, small daughter cysts, floating membranes and an air-fluid level within the cyst (white arrow). The diagnosis is an infected hydatid cyst (type Ⅳ).

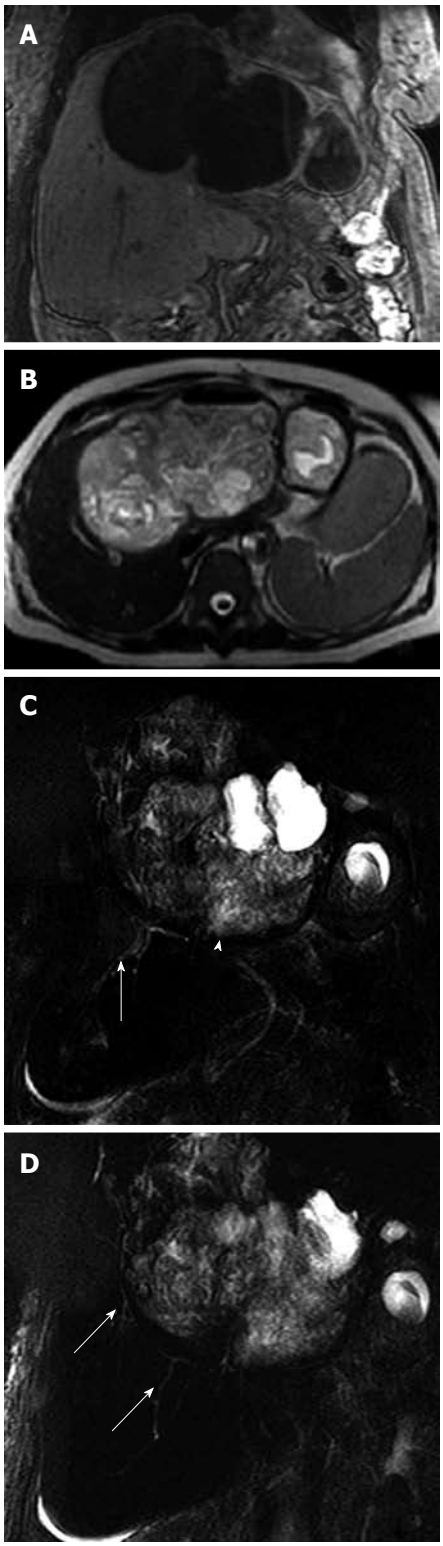

Figure 15 T1 coronal image and T2 axial image show a huge and multilocular hydatid cyst occupying the entire left lobe, partially the right lobe (A, B) and protruding into the hepatic hilum. Magnetic resonance cholangiopancreatography (MRCP) sequences show the compression of the common bile duct at the confluence and of the right hepatic duct (the white arrowhead) and the consequent intrahepatic biliary tree dilatation (the white arrows) (C, D).

Because the hydatid cyst contains viscous hydatid sand that consists of scolices, sodium chloride, proteins, glucose, ions, lipids, and polysaccharides, the ADC of the hydatid

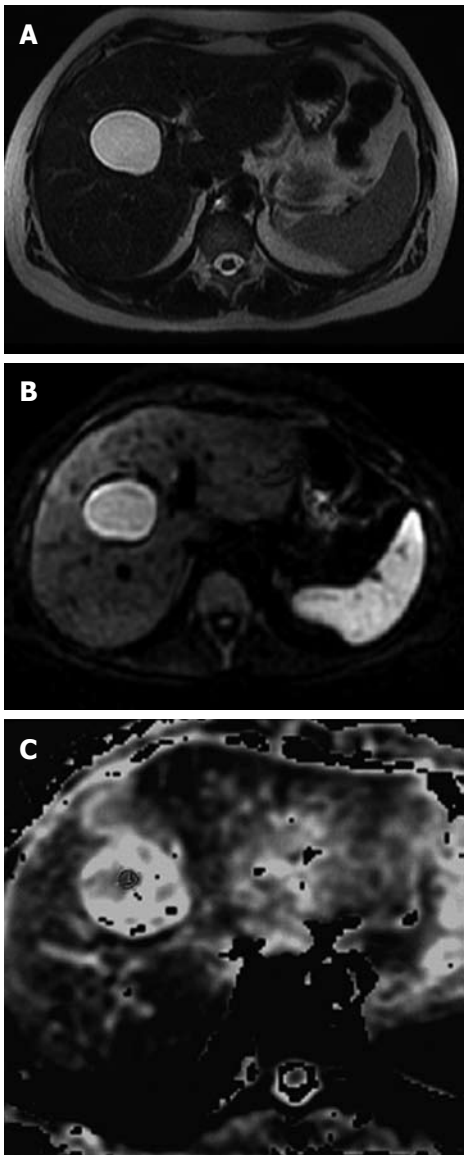

Figure 16 A type I hydatid cyst. A: Axial T2 weighted magnetic resonance image depicts a round cystic mass in the anterior segment of the right lobe, with no septa or solid portions; B: On the diffusion-weighted image the lesion exhibits high signal intensity (b = 1000); C: On apparent diffusion coefficient map, apparent diffusion coefficient value is 2.4 x 10^{-3}.

cyst is decreased; on the contrary the simple cyst has lower viscosity, hence the higher viscosity. In Inan's series the mean ADCs of the hydatid cysts was significantly lower (2.5 × 10^{-3} ± 0.9) than that of the simple cysts (3.5 × 10^{-3} ± 0.5) (Figure 16)[22-24].

In patients affected by hepatic hydatidosis, contrast enhanced magnetic resonance angiography may be useful in detecting hepatic venous outflow obstruction or thrombosis or invasion[25]; in these patients, pulmonary embolism may be a possible complication (Figure 17)[26].

CLASSIFICATION OF HYDATID DISEASE ON THE BASIS OF IMAGING

Hydatid disease is classified into four types on the basis of their radiologic appearance[27]:

Type Ⅰ: Simple cyst with non internal architecture

Hydatidosis appear at US as a well-defined anechoic mass with or without hydatid sand and septa. Unilocular cysts are considered to be an initial stage in the development of the parasite[28]. A solitary type I cyst may be difficult to distinguish from a simple epithelial cyst[8].

At CT, a type I appears as a well-defined water-attenuation mass; after injection of contrast material the septa and cyst wall enhance, a finding that helps differentiate type I from a simple liver cyst[13,19]. MR images are also similar to those of a simple liver cyst, including hypointensity on T1-weighted images and marked hyperintensity on T2-weighted images; a low signal intensity rim ("rim sign")[19,20], which is more evident on T2-weighted images, has been described as typical of hydatidosis, and it can be used to differentiate hydatid cysts from simple cysts; this finding represents the pericyst.

Recently the emerging role of DW MRI may play a decisive role in the differential diagnosis of hydatid liver disease and simple cysts[22-24] (Figures 1, 5 and 16).

Type Ⅱ: Cyst with daughter cysts and matrix

Daughter cysts are inside the mother cyst, usually arranged at the periphery[15,18]. Floating membranes or vesicles can be also seen in the cyst. Multiple daughter cysts are enclosed together looking like an echogenic solid lesion. (Figures 6, 10, 11 and 12)

Type Ⅱ may manifest as a well-defined fluid collection in a honeycomb pattern with multiple septa representing the walls of the daughter cysts, creating a "rosette" appearance[15]. Peripheral calcification may occur and involves the pericyst; it is easily detected in CT images as a curvilinear or ring-like structure. CT can distinguish the mother cyst: the average density attenuation of the mother cyst is higher than that of daughter cysts. At MR imaging, daughter cysts may appear hypointense or isointense relative to the maternal matrix on T1 and T2-weighted images[19,20].

Type Ⅲ: Calcified cyst

Type Ⅲ lesions are dead cysts with total calcification. At US calcified cysts show strong posterior shadowing, at CT they appear as round hyperattenuating areas, at MR they appear as hypointense areas (Figure 7).

Type Ⅳ

Hydatid complications include rupture and superinfection and may be seen in both type I and type Ⅱ. CT and MRI play a key role in recognizing the complications such as rupture and infection of cysts associated with hydatid disease.

Ruptures may occur in 50% of cases[6,9,11]; cyst rupture is mainly due to the degeneration of parasitic membranes. Cyst rupture is usually due to the degeneration of parasitic membranes, as a result of age, or a host defense mechanism[6,9,11]. The rupture may be contained, commu-

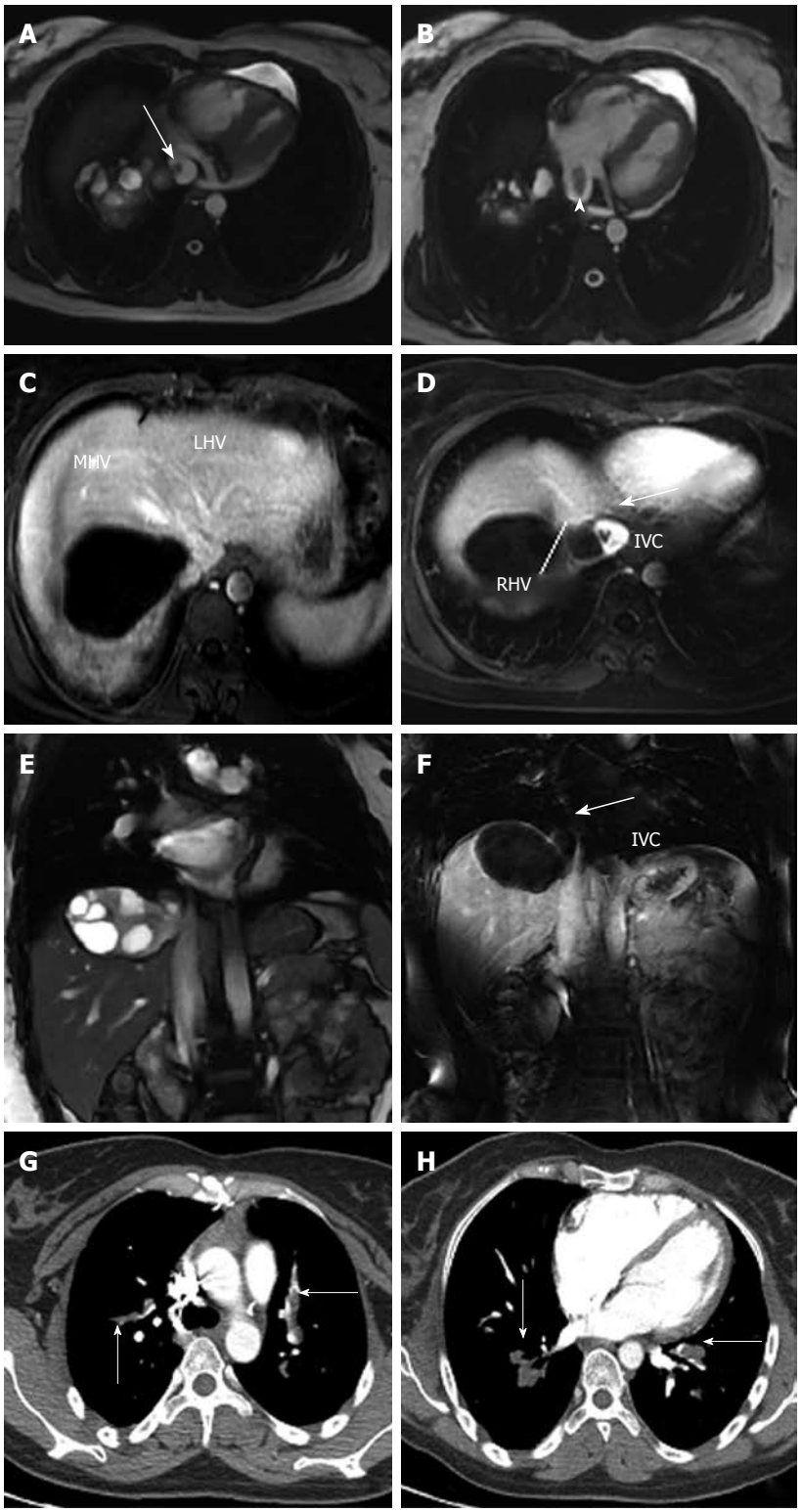

Figure 17 Hepatic hydatidosis in a 30-year-old woman who presented with short of breath, fatigue and edema to the lower limbs. Magnetic resonance (MR) steady-state-free-precession sequences (A, B, E) and MR angiography (C, D, F) images showed the hydatid cyst invading the right hepatic vein (RHV), protruding in the inferior vein cava (IVC) (the bold white arrow) and in the right atrium (the white arrowhead). The mid hepatic vein (MHV) and the left hepatic vein (LHV) were normally patent (C). The multidetector computed tomography-angiography revealed diffuse pulmonary parasitic embolism (the thin white arrows) (G, H).

nicating or direct.

Fissures in the cyst wall can be visualized at both CT and MR imaging. Perforation to the biliary tree has been reported in up to 90% of hydatid cysts[7,10,11]. Hydatid cysts may also rupture into pleural and peritoneal cavities. Up to 25% of ruptured cysts may become infected[10,11]. Signs of cyst infection include air-fluid or fluid-fluid levels[9,11] (Figures 8, 9 and 14).

CONCLUSION

Imaging plays a primary role in liver hydatidosis. It is used for diagnosis, for assessment of extension, for identification of possible complications, for classification and for monitoring the response to therapy. US, MDCT and MR have different roles depending on accuracy in depicting the different goals.

REFERENCES

1 **Eckert J**, Deplazes P. Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. *Clin Microbiol Rev* 2004; **17**: 107-135
2 **Czermak BV**, Akhan O, Hiemetzberger R, Zelger B, Vogel W, Jaschke W, Rieger M, Kim SY, Lim JH. Echinococcosis of the liver. *Abdom Imaging* 2008; **33**: 133-143
3 **Rozanes I**, Güven K, Acunaş B, Emre A. Cystic echinococcal liver disease: new insights into an old disease and an algorithm for therapy planning. *Cardiovasc Intervent Radiol* 2007; **30**: 1112-1116
4 **Filippou D**, Tselepis D, Filippou G, Papadopoulos V. Advances in liver echinococcosis: diagnosis and treatment. *Clin Gastroenterol Hepatol* 2007; **5**: 152-159
5 **Mortelé KJ**, Ros PR. Cystic focal liver lesions in the adult: differential CT and MR imaging features. *Radiographics* 2001; **21**: 895-910
6 **Pedrosa I**, Saíz A, Arrazola J, Ferreirós J, Pedrosa CS. Hydatid disease: radiologic and pathologic features and complications. *Radiographics* 2000; **20**: 795-817
7 **Polat P**, Kantarci M, Alper F, Suma S, Koruyucu MB, Okur A. Hydatid disease from head to toe. *Radiographics* 2003; **23**: 475-494; quiz 536-537
8 **Lewall DB**. Hydatid disease: biology, pathology, imaging and classification. *Clin Radiol* 1998; **53**: 863-874
9 **Mortelé KJ**, Segatto E, Ros PR. The infected liver: radiologic-pathologic correlation. *Radiographics* 2004; **24**: 937-955
10 **Lim JH**. Parasitic diseases in the abdomen: imaging findings. *Abdom Imaging* 2008; **33**: 130-132
11 **Turgut AT**, Altin L, Topçu S, Kiliçoğlu B, Aliinok T, Kaptanoğlu E, Karademir A, Koşar U. Unusual imaging characteristics of complicated hydatid disease. *Eur J Radiol* 2007; **63**: 84-93
12 **Scherer K**, Gupta N, Caine WP, Panda M. Differential diagnosis and management of a recurrent hepatic cyst: a case report and review of literature. *J Gen Intern Med* 2009; **24**: 1161-1165
13 **Coşkun A**, Oztürk M, Karahan OI, Erdogan N, Işin S, Güleç M. Alveolar echinococcosis of the liver: correlative color Doppler US, CT, and MRI study. *Acta Radiol* 2004; **45**: 492-498
14 **Hosch W**, Junghanss T, Werner J, Düx M. [Imaging methods in the diagnosis and therapy of cystic echinococcosis]. *Rofo* 2004; **176**: 679-687
15 **Turgut AT**, Akhan O, Bhatt S, Dogra VS. Sonographic spectrum of hydatid disease. *Ultrasound Q* 2008; **24**: 17-29
16 **Caremani M**, Lapini L, Caremani D, Occhini U. Sonographic diagnosis of hydatidosis: the sign of the cyst wall. *Eur J Ultrasound* 2003; **16**: 217-223
17 **Katranci N**, Elmas N, Yilmaz F, Mentes A. Correlative CT, MRI and histological findings of hepatic Echinococcus alveolaris: a case report. *Comput Med Imaging Graph* 1999; **23**: 155-159
18 **Marani SA**, Canossi GC, Nicoli FA, Alberti GP, Monni SG, Casolo PM. Hydatid disease: MR imaging study. *Radiology* 1990; **175**: 701-706
19 **Vilgrain V**. [Cystic lesions of the liver]. *Gastroenterol Clin Biol* 2001; **25**: B167-B177
20 **Kodama Y**, Fujita N, Shimizu T, Endo H, Nambu T, Sato N, Todo S, Miyasaka K. Alveolar echinococcosis: MR findings in the liver. *Radiology* 2003; **228**: 172-177
21 **Mendez Montero JV**, Arrazola Garcia J, Lopez Lafuente J, Antela Lopez J, Mendez Fernandez R, Saiz Ayala A. Fat-fluid level in hepatic hydatid cyst: a new sign of rupture into the biliary tree? *AJR Am J Roentgenol* 1996; **167**: 91-94
22 **Oruç E**, Yıldırım N, Topal NB, Kılıçturgay S, Akgöz S, Savcı G. The role of diffusion-weighted MRI in the classification of liver hydatid cysts and differentiation of simple cysts and abscesses from hydatid cysts. *Diagn Interv Radiol* 2010; **16**: 279-287
23 **Inan N**, Arslan A, Akansel G, Anik Y, Sarisoy HT, Ciftci E, Demirci A. Diffusion-weighted imaging in the differential diagnosis of simple and hydatid cysts of the liver. *AJR Am J Roentgenol* 2007; **189**: 1031-1036
24 **Chan JH**, Tsui EY, Luk SH, Fung AS, Yuen MK, Szeto ML, Cheung YK, Wong KP. Diffusion-weighted MR imaging of the liver: distinguishing hepatic abscess from cystic or necrotic tumor. *Abdom Imaging* 2001; **26**: 161-165
25 **Haliloglu N**, Erden A. Hepatic venous outflow obstruction after hydatid cyst surgery: evaluation with contrast enhanced magnetic resonance angiography. *Eur J Gastroenterol Hepatol* 2009; **21**: 776-780
26 **Menassa-Moussa L**, Braidy C, Riachy M, Tabet G, Smayra T, Haddad-Zebouni S, Ghossain M, Aoun N. [Hydatid disease diagnosed following a pulmonary embolism]. *J Mal Vasc* 2009; **34**: 354-357
27 **Precetti S**, Gandon Y, Vilgrain V. [Imaging of cystic liver diseases]. *J Radiol* 2007; **88**: 1061-1072
28 **Ermis F**, Dursun M, Akyüz F, Bakir B, Güven K. Hydatid cysts: three different stages on the same image. *Intern Med* 2007; **46**: 1673

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TOPIC HIGHLIGHT

Salvatore Gruttadauria, MD, PhD, Professor, *Series Editor*

Hepatic echinococcosis: Clinical and therapeutic aspects

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Abstract

Echinococcosis or hydatid disease (HD) is a zoonosis caused by the larval stages of taeniid cestodes belonging to the genus *Echinococcus*. Hepatic echinococcosis is a life-threatening disease, mainly differentiated into alveolar and cystic forms, associated with *Echinococcus multilocularis* (*E. multilocularis*) and *Echinococcus granulosus* (*E. granulosus*) infection, respectively. Cystic echinococcosis (CE) has a worldwide distribution, while hepatic alveolar echinococcosis (AE) is endemic in the Northern hemisphere, including North America and several Asian and European countries, like France, Germany and Austria. *E. granulosus* young cysts are spherical, unilocular vesicles, consisting of an internal germinal layer and an outer acellular layer. Cyst expansion is associated with a host immune reaction and the subsequent development of a fibrous layer, called the pericyst; old cysts typically present internal septations and daughter cysts. *E. multilocularis* has a tumor-like, infiltrative behavior, which is responsible for tissue destruction and finally for liver failure. The liver is the main site of HD involvement, for both alveolar and cystic hydatidosis. HD is usually asymptomatic for a long period of time, because cyst growth is commonly slow; the most frequent symptoms are fatigue and abdominal pain. Patients may also present jaundice, hepatomegaly or anaphylaxis, due to cyst leakage or rupture. HD diagnosis is usually accomplished with the combined use of ultrasonography and immunodiagnosis; furthermore, the improvement of surgical techniques, the introduction of minimally invasive treatments [such as puncture, aspiration, injection, re-aspiration (PAIR)] and more effective drugs (such as benzoimidazoles) have deeply changed life expectancy and quality of life of patients with HD. The aim of this article is to provide an up-to-date review of biological, diagnostic, clinical and therapeutic aspects of hepatic echinococcosis.

Key words: Hydatidosis; Cystic echinococcosis; Alveolar echinococcosis; Liver; PAIR; Albendazole; Treatment; Diagnosis

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INTRODUCTION

Echinococcosis or hydatid disease (HD) is a zoonosis caused by the larval stages of taeniid cestodes belonging to the genus *Echinococcus*. Six species of *Echinococcus* are known, but only four of them are responsible for human disease: *Echinococcus granulosus* (*E. granulosus*) (which causes cystic echinococcosis), *Echinoccus multilocularis* (*E. multilocularis*) (which causes alveolar echinococcosis), *E. vogeli* and *E. oligarthrus* (which cause polycystic echinococcosis). Recent studies have identified two new species, *E. felidis* and *E. shiquicus*, even if no data are available about their pathogenicity to humans.

Hepatic alveolar and cystic echinococcosis are both life-threatening diseases because of their medical and economical impact and their wide geographical distribution. Polycystic echinococcosis is, on the contrary, confined to Central and South America and only few cases of this condition have been reported in man[1,2].

The liver is the major site of HD involvement (about 75% of cases) both in the alveolar and in the cystic form[3]. This review is focused on the biological, epidemiological, clinical and therapeutic aspects of hepatic echinococcosis, with particular reference to *E. granulosus* cystic and *E. multilocularis* alveolar hydatidosis (Table 1).

HEPATIC ECHINOCOCCOSIS CAUSED BY *E. GRANULOSUS*

Cystic echinococcosis (CE) occurs as the result of infection by the larval stages of *E. granulosus*. CE is the most common form of HD, with a worldwide distribution, and it can be regarded as an emerging or re-emerging disease in several countries of the world.

E. granulosus: The parasite biology and life cycle

E. granulosus is a small tapeworm (length of 2-7 mm), whose body is made up by a mean number of three proglottids. There are ten distinct genetic types (G1-10) within *E. granulosus*, with a different geographical distribution, and these have been identified by molecular studies based on mitochondrial DNA sequences[4,5]: 2 sheep strains (G1-G2), 2 bovid strains (G3 and G5), a horse strain (G4), a camelid strain (G6), a pig strain (G7), a cervid strain (G8), a swine strain (G9) and a reindeer strain (G10).

The parasite life cycle involves dogs and other canids (coyotes, dingoes, red foxes) as definitive hosts and ungulates (sheeps, pigs, goats, horses) as intermediate hosts. Definitive hosts are infected by ingestion of offal containing hydatid cysts; the adult worms reside in the canine small bowel and their eggs or gravid proglottids are shed in the feces. After oral uptake of eggs by intermediate hosts, an oncosphere larva is released from the egg and penetrates the intestinal lamina propria, reaching the blood and lymph vessels which transport it to liver, lungs and other organs, where oncosphere larvae can develop into metacestodes (also known as hydatid cysts). Humans can accidentally become "aberrant" intermediate hosts, after ingestion of *Echinococcus* eggs excreted by infected carnivores.

Hydatid cysts are spherical, fluid-filled, unilocular vesicles, consisting of an internal cellular layer (germinal layer) and an outer acellular, laminated layer. The parasite cysts gradually expand and cause a granulomatous host reaction, followed by the development of a fibrous tissue layer (pericyst). Brood capsules and protoscolices bud from the germinal membrane; with time, internal septations and daughter cysts usually develop, modifying the unilocular morphology that is typical of young hydatid cysts. When definitive hosts ingest the cyst-containing organs of intermediate herbivore hosts, the *Echinococcus* life cycle can restart, as the protoscolices evaginate, attach to the intestinal mucosa and develop to adult stage in 30-80 d[6,7].

Molecular crosstalk between human host and parasite

Several studies have focused on the mechanisms of host-parasite interplay in CE.

The immune response to *E. granulosus* infection has been investigated through both clinical studies on patients with hydatidosis and sheep and mouse experimental models[8]. In the early stage of hydatid cyst development, a cell-mediated response involving macrophages, neutrophils and eosinophils is established[9-11]; antibody response is usually undetectable during the first weeks after infection, but IgE, IgG2 and IgG4 levels subsequently significantly increase[8]. Elevated levels of IgE for echinoccocal antigens are responsible for allergic reactions, such as itching, urticaria and anaphylactic shock[12].

E. granulosus induces both TH1 and TH2 response: elevated levels of TH1 cytokines, especially interferon-γ (IFN-γ)[13], but also TH2 cytokines, such as IL-4, IL-5 and IL-6, have been recorded in patients with HD[8,11]. The reason for this duplex cytokine secretion pattern is not known: TH1 and TH2 responses usually down-regulate each other, with a cross-inhibitory mechanism; it is assumed that the complex antigenic organization of *Echinococcus* may stimulate both T-cell subsets[14]. After chemotherapy treatment, surgical removal or natural death of a cyst, TH2 response quickly drops and TH1 response becomes predominant[15].

The metacestode attempt to escape from the host protective response involves complex and intriguing strategies aimed at modulating host response and protecting itself from elimination. *Echinococcus* tries, in fact, to minimize host reaction by exposing several immunomodulatory molecules to its host[16], interfering with complement

Table 1 Hydatid disease epidemiology and characteristics[6,7]

	Cystic echinococcosis	Alveolar echinococcosis
Causative agent	*E. granulosus*	*E. multilocularis*
Definitive hosts	Dogs and other canids (coyotes, dingoes, red foxes)	Red foxes, arctic foxes, coyotes, dogs and cats
Intermediate hosts	Ungulates	Rodents
Geographic distribution	Worldwide	North America, northern and central Eurasia
Worldwide incidence	1-200/100 000	0.03-1.2/100 000
Organ localization	Mainly liver and lungs	Mainly liver
Characteristics of hydatid lesions	Young cysts: spherical, fluid-filled, unilocular vesicles (diameter: 1-15 cm) Old cysts: internal septations, daughter cysts Three-layered structure: germinal layer, laminated layer, pericyst	Alveolar-like pattern, with numerous vesicles (< 1 mm up to 15 cm in diameter) and surrounding dense connective tissue, no cyst fluid, sometimes central necrosis
Type of growth in human organs	Concentric expansion	Tumor-like, infiltrative behaviour
Therapeutic options	Surgery, PT (especially PAIR), chemotherapy	Surgery, chemotherapy, EPIs

PTs: Percutaneous treatments; PAIR: Puncture, aspiration, injection, re-aspiration; EPIs : Endoscopic percutaneous interventions.

activity[17], altering leukocyte function[18] or using molecular mimicry[19].

Epidemiology and infection risk

E. granulosus has a worldwide distribution; the highest prevalence is recorded in the Mediterranean countries, Russia and China (in Sichuan Province human CE had a prevalence of 2.1% in 1997-1998[20]). Other hyperendemic areas are North and East Africa (prevalence > 3%), South America and Australia[21]. CE infection has re-emerged in certain parts of the world where it was once believed to be controlled, including Israel, Central Asia and Eastern Europe[21,22]. In Bulgaria the annual incidence of CE in children has increased from 0.7 per 100 000 in 1971-1982 to 5.4 in 1995[23]; in Kazakhstan the annual surgical incidence of CE over the whole country was below 1.4 per 100 000 inhabitants from 1988 until 1995 but has increased to 5.9 in 2000[24,25].

CE is typically a rural and occupational disease, since certain human activities, such as feeding dogs with the viscera of slaughtered livestock, increase the risk of infection. Humans acquire the parasite through fecal-oral contact, generally by handling infected domestic dogs or egg-containing feces. *Echinococcus* eggs adhere to the coat of animals, especially to hairs around the anus and on the muzzle and paws[26]. Eggs can also be ingested with contaminated water or vegetables; it is also possible that the contamination of surfaces and foodstuffs with *Echinococcus* eggs occurs *via* wind, flies, birds or beetles.

Some studies have evaluated several risk factors for infection: Campos-Bueno *et al*[27] studied a Spanish cohort of 127 CE infected patients, matched with 127 healthy controls, associating an increased risk for CE with having a higher number of dogs in the family and with dogs' ease of access to raw viscera of slaughtered animals. In Tibet a rise of infective risk was associated with nomadic life, age, playing with dogs, not protecting food from flies and raising yaks or sheep. Water wells were suspected to be a source of infection in African arid lands, where animals and humans often share the same water points[28].

Clinical aspects

After infection, humans are usually asymptomatic for a long period of time, since cyst growth is usually slow; in the liver the growth rate is variable, ranging from 1 mm to 5 mm in diameter per year. Most primary infections consist of a single cyst, but up to 20%-40% of infected people have multiple cysts. Presenting symptoms depend not only on the size and number of cysts, but also on the mass effect within the organ and upon surrounding structures. The signs and symptoms of liver hydatidosis include hepatomegaly, right/epigastric pain, nausea and vomiting. Cyst leakage or rupture may be responsible for systemic immunological responses, causing anaphylaxis; in one series, anaphylaxis complicated 10% of all intraperitoneal ruptures.

Cyst rupture in the peritoneal cavity may cause secondary CE, with the release of protoscolices and/or small cysts, which can grow to larger cysts.

Portal vein or bile duct obstruction, caused by the expanding cysts, may be responsible for segmental or lobar liver atrophy in the cyst-bearing lobes[29].

Other complications are rupture in the biliary tree with secondary cholangitis[30], biliary obstruction by daughter cysts, portal hypertension, ascites, intracystic or subphrenic abscess formation, development of a bronchobiliary fistula[31,32]. Hydatid cyst suppuration has been reported as occurring in 5% to 40% of patients[33]. Perforation in the biliary tree has been described in up to 90% of HD[34].

Diagnosis

Considering that the early stages of infection are usually asymptomatic, the diagnosis of liver CE may often be incidental, associated with an abdominal ultrasonography performed for other clinical reasons. In endemic areas, the presence of symptoms suggestive of CE in a person with a history of exposure to sheepdogs supports the suspicion of hydatidosis.

A non-invasive diagnosis of hepatic CE is usually accomplished with the combined use of radiologic imaging

and immunodiagnostic techniques. Abdominal ultrasonography is considered the gold standard for defining the number, site, dimensions and vitality of cysts[32,35,36] and it is also important to evaluate treatment options. A standardized ultrasonographic classification system for hepatic cysts has been developed by the World Health Organization (WHO)[37], in order to update the older Gharbi classification[38].

Ultrasonography is not always able to differentiate hydatid cysts from other space-occupying lesions, like tumors or liver abscesses, so that additional imaging techniques, such as magnetic resonance imaging (MRI) and CT scans, may be required. MRI should be preferred to CT, due to better visualization of liquid areas within the matrix[39]. MRI is also important for pre-surgical evaluation of CE.

Immunodiagnosis is useful to confirm a radiologic diagnosis and can also be an important tool for the follow-up after surgical or pharmacological treatment, even if not all patients with CE have a detectable immune response[40-42]. Serological test sensitivity is indeed inversely related to the degree of sequestration of the echinococcal antigens inside cysts; for instance, healthy, intact cysts can elicit a minimally detectable response, whereas previously ruptured or leaking cysts are associated with stronger immune responses.

Almost all traditional immunodiagnostic methods (e.g., Casoni intradermal test, complement fixation test, indirect hemagglutination test, indirect immunofluorescence antibody test, immunoelectrophoresis and latex agglutination test) have now been replaced by the enzyme-linked immunosorbent assay (ELISA) and/or immunoblotting[43]. In order to detect antibody response to parasite, several hydatid antigens have been extracted and used for serological diagnosis. Hydatid cyst fluid antigen B (AgB) and antigen 5 (Ag5) from *E. granulosus* are considered the most specific native antigens for the immunodiagnosis of CE[40,41], even though lack of sensitivity and specificity, technique standardization and cross-reactivity with antigens of other parasites[44-46] are major problems associated with immunodiagnosis of CE.

In doubtful cases, for example undetectable anti-*Echinococcus* antibodies in patients with small lesions resembling hydatid cysts or in patients whose hepatic cysts cannot be differentiated from liver abscess or neoplasms, ultrasonography-guided fine needle puncture may represent an additional diagnostic option. The demonstration of protoscolices or hydatid membranes or echinococcal antigens/DNA in the aspirated cyst fluid can confirm, in fact, the diagnosis of CE. Anthelmintic coverage is important to minimize the risk of secondary CE: albendazole should be recommended for 4 d before the procedure and should be continued for at least 1 mo after having punctured a lesion recognized as an *E. granulosus* cyst[32,47]. Detection of parasite-specific IgE has no significant diagnostic advantages, even if eosinophilia is often present after rupture/leakage of the cyst[48].

Treatment

The goals of hepatic hydatid cyst treatment are a complete elimination of the parasite and prevention of recurrence, minimizing mortality and morbidity risk. In order to achieve these aims, it is essential to choose the most appropriate treatment with regard to disease-specific characteristics (cyst number, size, site, presence of cystobiliary communication), to patient clinical conditions, availability of an experienced surgeon or an interventional radiologist.

Three therapeutic modalities are available to treat hepatic CE: chemotherapy, surgery (with open or laparoscopic approach) and percutaneous treatments (PTs). A stage-specific approach is recommended[49].

Surgery: Until the 1980s, surgery was the only therapeutic option for patients with CE. Surgery is still the first choice for large CE2-CE3b cysts with multiple daughter cysts or for single superficial cysts, considering the likelihood of spontaneous or traumatic rupture, when PT is not available. Presence of complicated cysts, e.g., infected cysts or cysts communicating with the biliary tree, and cysts exerting pressure on other vital organs, are other indications for surgical approach. Surgery is contraindicated in patients whose preexisting medical conditions put them at risk or in patients having inactive asymptomatic cysts or multiple cysts which are difficult to access. If feasible, surgical removal of hydatid cysts has the best chance to completely remove cysts and to immediately cure CE.

Surgical options can be divided into radical (pericystectomy) and conservative approaches (for instance unroofing or capitonnage)[50-53]. Radical procedures are associated with a lower risk of recurrence, but also with a higher operative risk; conservative procedures, on the contrary, are easier to perform but have a higher likelihood of recurrence. Recurrence is usually due to either inadequate cyst removal or to previously undetected cysts; reported recurrence rates range from 2% to 25%[54].

Whichever technique is used, a benzimidazole (BMZ) agent is usually used to reduce the risk of anaphylaxis and secondary CE[55]. BMZ is administered from 1 d before surgery to 1 mo after surgery but, again, no conclusive data about the best timing are available. Major complications of surgery are postoperative hemorrhage, cholangitis, sepsis and fistulae formation. Operative mortality varies from 0.5% to 4%[55].

Percutaneous treatments: PTs of hepatic CE can aim at the destruction of the germinal layer [puncture, aspiration, injection, re-aspiration (PAIR)] or the evacuation of the entire endocyst ("modified catheterization technique").

PAIR is an acronym that stands for "puncture, aspiration, injection, re-aspiration". PAIR consists of four steps: (1) percutaneous puncture of the cyst using ultrasound guidance; (2) aspiration of the cyst fluid; (3) injection of a protoscolicidal agent (e.g., 95% ethanol or 20% NaCl) for at least 15 min; and (4) re-aspiration of the fluid[37,56].

PAIR is indicated for CE1 and CE3a cysts > 5 cm[49,56]; CE2 and CE3b cysts treated by PAIR tend to relapse. PAIR has also been used for patients who refused surgery

or relapsed after surgical treatment. It is contraindicated for inaccessible or superficially located liver cysts and for inactive or calcified cystic lesions. The possibility of secondary echinococcosis can be minimized by concurrent treatment with benzimidazoles; indeed, combined treatment (PAIR plus albendazole) may yield better results than those of either chemotherapy or PAIR alone[57,58]. The length of administration of chemotherapy with albendazole usually ranges between 4 h before and 1 mo after PAIR, in order to reduce the risk of disease recurrence and intraperitoneal seeding of infection. PAIR must be avoided in patients with cystobiliary communications, to prevent the risk of sclerosing cholangitis.

Chemotherapy: Mebendazole (MBZ) and albendazole (ABZ) are the BMZ agents used for the treatment of hepatic CE. They interfere with the absorption of glucose through the wall of the parasite, causing glycogen depletion and degenerative changes in echinococcal mitochondria and endoplasmic reticulum. BMZ may be favorably used alone for the treatment of small (< 5 cm) CE1-CE3a liver cysts[59] or for inoperable patients; BMZs are also usually associated with PAIR or surgery to prevent secondary CE[55]. BMZs are not indicated for the treatment of inactive or calcified asymptomatic cysts, unless they are complicated lesions[49].

Both ABZ and MBZ are effective, but ABZ is considered the drug of choice, because it is more active *in vitro* and it has a better gastrointestinal absorption and bioavailability[60,61]. The usual dose of orally-administered ABZ is 10-15 mg/kg per day in two divided doses; if MBZ, the daily dose is 40-50 mg/kg in three divided doses. Treatment with BMZ should be administered continuously, for 3-6 mo[49].

Clinical and radiographic improvement (in most studies defined as > 25% reduction in cyst size, membrane separation, or cyst calcification[62]) is quite frequent and is favorably influenced by the duration of treatment. Unfortunately, complete cure (i.e., cyst disappearance) only occurs in approximately a third of patients treated with BMZ alone and, interestingly, the number of patients with cure does not significantly increase by extending the duration of treatment[60]. A recent systematic review[63] has confirmed that the size and stage of cysts are the key factors to evaluate the likelihood of response to chemotherapy.

Usual adverse effects include nausea, hepatotoxicity, neutropenia and occasionally alopecia. Thus, all patients should have regular monitoring of leukocyte counts and liver function tests. Contraindications to chemotherapy include pregnancy, chronic hepatic diseases and bone marrow depression.

Praziquantel has been used (40 mg/kg once a week) with ABZ for combined treatment of CE; this therapeutic association seems to be more effective than ABZ alone[64].

For uncomplicated CE4 and CE5 cysts a "watch and wait" strategy is currently advised[49].

HEPATIC ALVEOLAR ECHINOCOCCOSIS CAUSED BY *E. MULTILOCULARIS*

Hepatic alveolar echinococcosis (AE) results from infection by the larval forms of *E. multilocularis*. The echinococcal metacestode develops in the liver and is characterized by an alveolar structure, made up by several vesicles surrounded by large granulomas. Human AE is a severe and emerging disease, whose prognosis is bleak in absence of treatment or if it is not diagnosed at an early stage of disease.

E. multilocularis: The parasite life cycle

E. multilocularis is a small cestode (1.2-4.5 mm), whose definitive hosts are wild carnivores such as red fox and arctic fox (sylvatic cycle) or domestic dogs and cats (synanthropic cycle). The adult tapeworms, whose bodies are characterized by a mean number of five proglottids, reside in the small bowel of their definitive hosts, where gravid proglottids release eggs which are passed in the feces. Intermediate hosts, usually small rodents, or aberrant hosts such as humans, become infected by ingestion of embryonated eggs. Human infection can happen through direct contact with the definitive host or it can be indirect, through contamination of food or water with parasite eggs[7,65]. The echinococcal metacestode develops in the liver and is characterized by an alveolar structure, made up by several vesicles whose diameter varies from < 1 mm up to 15-20 cm[47,65]. Each vesicle has a wall structure similar to that of the *E. granulosus* cyst, consisting of a germinal and a laminated layer[66]. Brood capsules or protoscolices are only occasionally seen and lesions may be complicated by central necrosis, producing a cavity or pseudocyst after liquidization. Small cysts are surrounded by a dense connective tissue and they usually do not contain fluid but instead a semisolid matrix[6].

Host-parasite interaction

E. multilocularis is able to elicit a strong cellular immune response: in the liver, parasitic lesions appear to be surrounded by large granulomas made up by macrophages, T-lymphocytes and myofibroblasts[67-69]. Observations in humans and experiments with rodents have shown that cellular immunity, related to TH1 cytokine profile, has a crucial role in host defense against the parasite[70]. IL-12, a key factor in the induction of TH1 profile, has been shown to inhibit, in mice, the development of alveolar lesions, leading to the formation of abortive parasitic vesicles surrounded by fully efficient periparasitic immune cell infiltration and fibrosis[71]. In mice treated with IFN-γ, a typical TH1 cytokine, a partial reduction in larval growth has been observed[72]. In contrast, a TH2 cytokine profile has been associated with disease progression: high levels of IL-5 and IL-10 have been detected in serum of patients with progressive disease, compared with individuals with abortive forms[73-76]. As in the case of *E. granulosus*, several mechanisms have been proposed

to explain *E. multilocularis* avoidance from host-protective responses, including antigenic disguise[77], immunomodulation[78-80], molecular mimicry[81], antigen and DNA polymorphism[82,83].

Epidemiology and infection risk

Data on human AE are difficult to be evaluated due to its low prevalence[21], which does not allow a reliable recognition of temporal developments or differences in spatial distribution. The long asymptomatic period also makes it difficult to determine time and place of infection[84]. *E. multilocularis* is endemic in the Northern hemisphere, including North America (Alaska, Canada), Asia (some of the newly independent states of the former Soviet Union[85], China[86] and Japan) and some European countries[87] (mainly France, Switzerland, Austria, Germany)[21,22]. In endemic areas, annual incidence of AE ranges from 0.03 to 1.2/100 000 inhabitants[88,89]. Increasing fox population, increased fox encroachment into urban areas and *E. multilocularis* spillover from wild carnivores to domestic hosts, are all factors that may explain *E. multilocularis* spreading from endemic areas to previously non-endemic European countries[21,90].

Considering the parasite life cycle, exposure of humans to echinococcal eggs may be influenced by occupational and behavioral factors. Hunters, trappers and persons who work with fox fur should be more frequently exposed to *E. multilocularis* eggs, but there is no evidence that these groups are at increased risk[91,92].

Clinical aspects

Slow larval growth results in an asymptomatic phase of several years (5-15 years). Initially, the liver, usually the right lobe, is the organ where the metacestodes establish themselves; then, later in the infection, it is possible to find blood metastasis to lung, brain, bones and local extension of the lesion (abdomen, retroperitoneum, diaphragm)[66]. First symptoms are usually vague: patients may complain of fatigue, weight loss or may have hepatomegaly. One third of them have cholestatic jaundice; one third present with abdominal pain[54,66,93]. In advanced stages, liver failure usually occurs and it is frequently associated with portal hypertension, ascites and splenomegaly. The prognosis in untreated or inadequately treated patients with AE is poor. Treatment has radically changed average life expectancy at diagnosis from 3 years in the 1970s to 20 years in 2005[94].

Diagnosis

As for CE, AE diagnosis is based on clinical and epidemiologic findings, imaging techniques, nucleic acid detection and serology.

Among the imaging techniques, ultrasonography is the method of choice to identify hydatid lesions: ultrasound (US) typical aspect shows a pseudotumoral mass, with irregular limits and scattered calcification, where hypoechogenic and hyperechogenic areas are juxtaposed; central necrosis may give to the mass the appearance of a cystic-like structure, surrounded by a hyperechogenic ring[95,96]. Color doppler may be useful to evaluate biliary and vascular infiltration. Abdominal CT gives further anatomical details and information about the lesion pattern of calcification[65]. MR imaging is the best standard to study the invasion of adjacent structures and may help in unclear cases[97]. Pre-surgical percutaneous cholangiography is important to assess the presence of communication between the biliary tree and the alveolar lesions[96]; it is also fundamental to exclude extra-hepatic involvement, through pulmonary and cerebral radiological examination.$^{(18F)}$Fluorodeoxyglucose positron emission tomography (FDG-PET) scanning gives indirect information on the parasite metabolic activity, especially if combined with MRI or CT scan; if negative, this finding does not mean that the parasite is not viable but that there is a suppressed periparasitic inflammatory activity[98].

WHO classification of AE is based on imaging findings and it is useful to have an internationally recognized, uniform standard for disease diagnosis and treatment strategies. The WHO-IWGE PNM classification system[65,99] is similar to tumor TNM classification: "P" refers to the extent of parasite localization inside the liver, "N" establishes the involvement of neighboring organs, "M" evaluates the absence (M0) or presence (M1) of distant metastasis, after having performed a chest X-ray and a cerebral CT.

As in CE, immunodiagnosis has a complementary role to other procedures, not only in primary diagnosis but also for follow-up of patients after surgical treatment or chemotherapy[100,101] and for the specific differential diagnosis between AE and CE in those regions where the diseases are co-endemic[102,103]. Immunodiagnosis (with indirect hemoagglutination test or ELISA) is more reliable for the diagnosis of AE than for CE, because more specific antigens are available. For example, the Em2plus-ELISA, which is a mixture of affinity purified *E. multilocularis* metacestode antigens (Em2-antigen) and a recombinant antigen (Em Ⅱ/3-10), has shown a great sensitivity and specificity[104], but it is not able to discriminate between active and inactive lesions; in fact, Em2-ELISA may be positive for years after spontaneous or pharmacological-induced dying out of the metacestode in patients with calcified lesions, because the Em2 antigen main source is the laminated layer of the parasite which obviously persists in these inactive lesions. Surgical removal of the dried-out lesion results in an immediate seroconversion to negative anti-Em2 antibodies[105,106]. Considering that the protoscolex is the most active component of echinococcal tissues, protoscolex antigens Em16 and Em18 have been isolated and used for immunoblot tests, in order to discriminate between active and inactive lesions[107]; recombinant (r) Em18 appears to be a promising immunodiagnostic tool for serological differentiation between AE and CE[107,108]. Combining US and serological data, it is possible to classify seropositive patients into three groups: patients with active hepatic lesions, patients with calcified lesions and patients with no

evidence of hepatic lesions[49]. The latter cases are a consequence of immune system pressure, which can cause larval degeneration and death, so that the only radiological sign of the host-parasite interaction may be the US finding of calcifications[109].

Some studies have shown that patients with AE have high levels of IgG1 and IgG4 antibodies and that after treatment they usually become seronegative for IgG4 antibodies[110-113]; IgG4 antibody reappearance can be considered a warning sign of disease reactivation.

Liver needle biopsy can be performed in uncertain cases and it can confirm AE diagnosis if histopathological examination identifies the presence of alveolar vesicles. RT-PCR on liver specimens, obtained by biopsy or surgery, has been used to assess parasite viability, while PCR can detect *E. multilocularis* DNA. These tests have a good positive predictive value, but a negative result does not exclude parasite activity and parasite presence in the liver, respectively[114].

Treatment

The key concept of AE treatment is to adopt a multidisciplinary approach to disease. Surgery and chemotherapy are the cornerstones of AE treatment and, as for CE, a stage-specific approach is recommended[49].

Surgery: Surgery is the first-choice option in all operable patients. Radical resection of the entire hepatic parasitic lesions is the only curative procedure, even though it is often difficult to achieve because of echinococcal dissemination into host tissues. Palliative liver surgery is almost always contraindicated, because it does not offer advantages when compared with conservative treatment[115,116]. Preoperative evaluation is important to establish lesions full resectability; WHO-IWGE PNM classification estimates quite well the likelihood to achieve radical resection[99].

Liver transplant (LT) has been employed in otherwise terminal cases[117]. Indications for LT are the presence of severe liver failure or recurrent life-threatening cholangitis and the inability to perform a radical liver resection. The absence of extra-hepatic AE localizations is mandatory for LT[49].

BMZ chemotherapy should be carried out for at least 2 years after surgery and patients should be monitored for at least 10 years, because of the risk of recurrence: in fact, unrecognized or invisible parasites can re-grow, even after some years, especially in post-LT immunosuppressed patients[118].

Chemotherapy: Inoperable AE patients should receive continuous BMZ treatment for life; moreover, long-term BMZ administration (at least 2 years) is mandatory after surgical treatment. Pre-surgical BMZ therapy is advised only in the case of LT. ABZ is given orally at a dosage of 10-15 mg/kg per day, in two divided doses; if it is not tolerated, MBZ may be given at daily doses of 40-50 mg/kg per day, split into three divided doses with fat-rich meals[49]. Conventional and liposomal amphotericin B has been used in patients who did not tolerate BMZ[119]. In a recent study nitazoxanide has not shown any efficacy for AE treatment[120].

Therapy with BMZ has resulted in an increased 10-year survival rate of approximately 80% (6%-25% in untreated historical controls)[121]. BMZs are parasitostatic, not parasiticidal: after several years of BMZ treatment, in the absence of progression of AE lesions, it is possible to discuss whether treatment should be continued or not. Decision-making should be supported by the evaluation of parasite viability, usually by PET-CT[98], and serum specific antibodies[101,102]. These tools may also be useful for the follow-up after BMZ withdrawal.

All AE patients should be monitored by US at frequent intervals and CT and/or MRI at intervals of 2-3 years, to evaluate disease recurrence or progression[49].

Endoscopic percutaneous interventions: Interventional procedures may be considered in inoperable patients in the presence of complications such as liver abscesses, jaundice due to biliary duct obstruction, portal vein thrombosis or bleeding esophageal varices associated with portal hypertension[96]. EPIs with BMZ avoid palliative surgery and may improve the patient life expectancy and quality of life.

CONCLUSION

Liver echinococcosis is a severe, neglected, often misdiagnosed disease; both AE and CE may be considered emerging public health problems, since CE is endemic in several countries in the world and AE is one of the most lethal helminthic diseases.

The last years have been characterized by significant advances in the knowledge of *Echinococcus* biology and interaction with the immune system; the development of more specific and sensitive immunological tests and the introduction of PCR for detection of parasite nucleic acid have increased the range of diagnostic tools. Furthermore, the improvement in surgical techniques, the introduction of effective drugs (e.g., BMZ) and minimally invasive treatments (e.g., PAIR) have deeply changed the life expectancy and quality of life of patients with HD.

Despite diagnostic and therapeutic progress, many unresolved problems are still waiting for a solution; for instance, there is a need for prevention programs able to monitor and control parasite spreading. Additionally, randomized, controlled trials comparing different therapeutic options, especially for CE, are urgently required, in order to provide new evidence to guide treatment decision-making.

REFERENCES

1 **Rausch R,** D'Alesandro A. The epidemiology of echinococcosis caused by Echinococcus oligarthrus and E vogeli in the neotropics. In: Craig P, Pawlowksi Z. Cestode zoonoses: echinococcosis and cysticercosis. Amsterdam: IOS Press, 2002: 107-130

2 **D'Alesandro A.** Polycystic echinococcosis in tropical America: Echinococcus vogeli and E oligarthrus. *Acta Trop* 1997; **67**: 43-65
3 **Polat P**, Kantarci M, Alper F, Suma S, Koruyucu MB, Okur A. Hydatid disease from head to toe. *Radiographics* 2003; **23**: 475-494; quiz 536-537
4 **McManus DP**, Thompson RC. Molecular epidemiology of cystic echinococcosis. *Parasitology* 2003; **127** Suppl: S37-S51
5 **Thompson RC**, McManus DP. Towards a taxonomic revision of the genus Echinococcus. *Trends Parasitol* 2002; **18**: 452-457
6 **Thompson RC,** McManus DP. Aetiology: parasites and lifecycles. In: Eckert J, Gemmell M, Meslin FX, Pawlowski Z. WHOI/OIE manual on echinococcosis in humans and animals: a public health problem of global concern. Paris: World Organisation for Animal Health, 2001: 1-19
7 **Thompson RC.** Biology and systematics of Echinococcus. In: Thompson RCA, Lymbery AJ. The biology of Echinococcus and hydatid disease. Wallingford: CAB International, 1995: 1-50
8 **Zhang W**, Li J, McManus DP. Concepts in immunology and diagnosis of hydatid disease. *Clin Microbiol Rev* 2003; **16**: 18-36
9 **Archer GT**, Robson JE, Thompson AR. Eosinophilia and mast cell hyperplasia induced by parasite phospholipid. *Pathology* 1977; **9**: 137-153
10 **Riganò R**, Profumo E, Siracusano A. New perspectives in the immunology of Echinococcus granulosus infection. *Parassitologia* 1997; **39**: 275-277
11 **Riganò R**, Profumo E, Teggi A, Siracusano A. Production of IL-5 and IL-6 by peripheral blood mononuclear cells (PBMC) from patients with Echinococcus granulosus infection. *Clin Exp Immunol* 1996; **105**: 456-459
12 **Vuitton DA**. Echinococcosis and allergy. *Clin Rev Allergy Immunol* 2004; **26**: 93-104
13 **Touil-Boukoffa C**, Bauvois B, Sancéau J, Hamrioui B, Wietzerbin J. Production of nitric oxide (NO) in human hydatidosis: relationship between nitrite production and interferon-gamma levels. *Biochimie* 1998; **80**: 739-744
14 **McManus DP,** Bryant C. Biochemistry, physiology and molecular biology of Echinococcus. In: RCA Thompson and AJ Lymbery, editors. The biology of Echinococcus and hydatid disease. Wallingford: CAB International, 1995: 355-410
15 **Riganò R**, Profumo E, Ioppolo S, Notargiacomo S, Ortona E, Teggi A, Siracusano A. Immunological markers indicating the effectiveness of pharmacological treatment in human hydatid disease. *Clin Exp Immunol* 1995; **102**: 281-285
16 **Vuitton DA**. The ambiguous role of immunity in echinococcosis: protection of the host or of the parasite? *Acta Trop* 2003; **85**: 119-132
17 **Ferreira AM**, Würzner R, Hobart MJ, Lachmann PJ. Study of the in vitro activation of the complement alternative pathway by Echinococcus granulosus hydatid cyst fluid. *Parasite Immunol* 1995; **17**: 245-251
18 **Annen JM**, Köhler P, Eckert J. Cytotoxicity of Echinococcus granulosus cyst fluid in vitro. *Z Parasitenkd* 1981; **65**: 79-88
19 **Dixon JB,** Jenkins P. Immunology of mammalian metacestode infections. I. Antigens, protective immunity and immunopathology. *Helm Abstr* 1995; **64**: 532-542
20 **Wang Q**, Qiu JM, Schantz P, He JG, Ito A, Liu FJ. Investigation of risk factors for development of human hydatidosis among households raising livestock in Tibetan areas of western Sichuan province. *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi* 2001; **19**: 93-96
21 **Eckert J,** Schantz P, Gasser R. Geographic distribution and prevalence. In: Eckert J, Gemmell MA, Meslin FX, Pawlowski ZS, editors. WHOI/OIE manual on echinococcosis in humans and animals: a public health problem of global concern. Paris: World Organisation for Animal Health, 2001: 100-141
22 **Ito A**, Urbani C, Jiamin Q, Vuitton DA, Dongchuan Q, Heath DD, Craig PS, Zheng F, Schantz PM. Control of echinococcosis and cysticercosis: a public health challenge to international cooperation in China. *Acta Trop* 2003; **86**: 3-17
23 **Todorov T**, Boeva V. Human echinococcosis in Bulgaria: a comparative epidemiological analysis. *Bull World Health Organ* 1999; **77**: 110-118
24 **Shaikenov BS,** Torgerson PR. Distribution of Echinococcus multilocularis in Kazakhstan. In: Craig P, Pawlowski Z. Cestode zoononoses: echinococcosis and cysticercosis, an emergent and global problem. Amsterdam: IOS Press, 2002: 299-307
25 **Shaikenov BS**, Vaganov TF, Torgerson PR. Cystic echinococcosis in Kazakhstan: an emerging disease since independence from the Soviet Union. *Parasitol Today* 1999; **15**: 172-174
26 **Moro PL**, Cavero CA, Tambini M, Briceño Y, Jiménez R, Cabrera L. Identification of risk factors for cystic echinococcosis in a peri-urban population of Peru. *Trans R Soc Trop Med Hyg* 2008; **102**: 75-78
27 **Campos-Bueno A**, López-Abente G, Andrés-Cercadillo AM. Risk factors for Echinococcus granulosus infection: a case-control study. *Am J Trop Med Hyg* 2000; **62**: 329-334
28 **Macpherson CNL.** Epidemiology of Echinococcus granulosus in transhumant situations. In: Eckert J, Gemmell MA, Meslin FX, Pawlowski ZS, editors. WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern. Paris: World Organisation for Animal Health, 2001: 156-163
29 **Prousalidis J**, Tzardinoglou E, Kosmidis C, Katsohis K, Aletras O. Surgical management of calcified hydatid cysts of the liver. *HPB Surg* 1999; **11**: 253-259
30 **Atli M**, Kama NA, Yuksek YN, Doganay M, Gozalan U, Kologlu M, Daglar G. Intrabiliary rupture of a hepatic hydatid cyst: associated clinical factors and proper management. *Arch Surg* 2001; **136**: 1249-1255
31 **Kammerer WS**, Schantz PM. Echinococcal disease. *Infect Dis Clin North Am* 1993; **7**: 605-618
32 **Pawlowski Z,** Eckert J, Vuitton DA, Ammann RW, Kern P, Craig PS. Echinococcosis in humans: clinical aspects, diagnosis and treatment. In: Eckert J, Gemmell MA, Meslin FX, Pawlowski Z, editors. WHO/OIE Manual on Echinococcosis in humans and animals. Paris: Office International des Epizooties, 2001: 20-71
33 **Ergüney S**, Tortum O, Taspinar AH, Ertem M, Gazioğlu E. [Complicated hydatid cysts of the liver]. *Ann Chir* 1991; **45**: 584-589
34 **Pedrosa I**, Saíz A, Arrazola J, Ferreirós J, Pedrosa CS. Hydatid disease: radiologic and pathologic features and complications. *Radiographics* 2000; **20**: 795-817
35 **Cohen H**, Paolillo E, Bonifacino R, Botta B, Parada L, Cabrera P, Snowden K, Gasser R, Tessier R, Dibarboure L, Wen H, Allan JC, Soto de Alfaro H, Rogan MT, Craig PS. Human cystic echinococcosis in a Uruguayan community: a sonographic, serologic, and epidemiologic study. *Am J Trop Med Hyg* 1998; **59**: 620-627
36 **Shambesh MA**, Craig PS, Macpherson CN, Rogan MT, Gusbi AM, Echtuish EF. An extensive ultrasound and serologic study to investigate the prevalence of human cystic echinococcosis in northern Libya. *Am J Trop Med Hyg* 1999; **60**: 462-468
37 **WHO Informal Working Group.** International classification of ultrasound images in cystic echinococcosis for application in clinical and field epidemiological settings. *Acta Trop* 2003; **85**: 253-261
38 **Gharbi HA**, Hassine W, Brauner MW, Dupuch K. Ultrasound examination of the hydatic liver. *Radiology* 1981; **139**: 459-463
39 **Hosch W**, Junghanss T, Stojkovic M, Brunetti E, Heye T, Kauffmann GW, Hull WE. Metabolic viability assessment of cystic echinococcosis using high-field 1H MRS of cyst con-

tents. *NMR Biomed* 2008; **21**: 734-754
40 **Zhang W**, McManus DP. Recent advances in the immunology and diagnosis of echinococcosis. *FEMS Immunol Med Microbiol* 2006; **47**: 24-41
41 **Ito A**. Serologic and molecular diagnosis of zoonotic larval cestode infections. *Parasitol Int* 2002; **51**: 221-235
42 **Gottstein B**. Molecular and immunological diagnosis of echinococcosis. *Clin Microbiol Rev* 1992; **5**: 248-261
43 **Craig PS**, Rogan MT, Campos-Ponce M. Echinococcosis: disease, detection and transmission. *Parasitology* 2003; **127** Suppl: S5-20
44 **Liu D**, Rickard MD, Lightowlers MW. Assessment of monoclonal antibodies to Echinococcus granulosus antigen 5 and antigen B for detection of human hydatid circulating antigens. *Parasitology* 1993; **106** (Pt 1): 75-81
45 **Ortona E**, Riganò R, Margutti P, Notargiacomo S, Ioppolo S, Vaccari S, Barca S, Buttari B, Profumo E, Teggi A, Siracusano A. Native and recombinant antigens in the immunodiagnosis of human cystic echinococcosis. *Parasite Immunol* 2000; **22**: 553-559
46 **Poretti D**, Felleisen E, Grimm F, Pfister M, Teuscher F, Zuercher C, Reichen J, Gottstein B. Differential immunodiagnosis between cystic hydatid disease and other cross-reactive pathologies. *Am J Trop Med Hyg* 1999; **60**: 193-198
47 **Hira PR**, Shweiki H, Lindberg LG, Shaheen Y, Francis I, Leven H, Behbehani K. Diagnosis of cystic hydatid disease: role of aspiration cytology. *Lancet* 1988; **2**: 655-657
48 **Khabiri AR**, Bagheri F, Assmar M, Siavashi MR. Analysis of specific IgE and IgG subclass antibodies for diagnosis of Echinococcus granulosus. *Parasite Immunol* 2006; **28**: 357-362
49 **Brunetti E**, Kern P, Vuitton DA. Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. *Acta Trop* 2010; **114**: 1-16
50 **Erdener A**, Ozok G, Demircan M. Surgical treatment of hepatic hydatid disease in children. *Eur J Pediatr Surg* 1992; **2**: 87-89
51 **Perdomo R**, Alvarez C, Monti J, Ferreira C, Chiesa A, Carbó A, Alvez R, Grauert R, Stern D, Carmona C, Yarzabal L. Principles of the surgical approach in human liver cystic echinococcosis. *Acta Trop* 1997; **64**: 109-122
52 **Cirenei A**, Bertoldi I. Evolution of surgery for liver hydatidosis from 1950 to today: analysis of a personal experience. *World J Surg* 2001; **25**: 87-92
53 **Dziri C**, Haouet K, Fingerhut A. Treatment of hydatid cyst of the liver: where is the evidence? *World J Surg* 2004; **28**: 731-736
54 **Ammann RW**, Eckert J. Cestodes. Echinococcus. *Gastroenterol Clin North Am* 1996; **25**: 655-689
55 **Arif SH**, Shams-Ul-Bari NA, Zargar SA, Wani MA, Tabassum R, Hussain Z, Baba AA, Lone RA. Albendazole as an adjuvant to the standard surgical management of hydatid cyst liver. *Int J Surg* 2008; **6**: 448-451
56 **Informal Working Group on Echinococcosis.** PAIR: Puncture, Aspiration, Injection, Re-Aspiration. An Option for the Treatment of Cystic Echinococcosis. Geneva: WHO, 2003
57 **Khuroo MS**, Dar MY, Yattoo GN, Zargar SA, Javaid G, Khan BA, Boda MI. Percutaneous drainage versus albendazole therapy in hepatic hydatidosis: a prospective, randomized study. *Gastroenterology* 1993; **104**: 1452-1459
58 **Smego RA**, Bhatti S, Khaliq AA, Beg MA. Percutaneous aspiration-injection-reaspiration drainage plus albendazole or mebendazole for hepatic cystic echinococcosis: a meta-analysis. *Clin Infect Dis* 2003; **37**: 1073-1083
59 **Vutova K**, Mechkov G, Vachkov P, Petkov R, Georgiev P, Handjiev S, Ivanov A, Todorov T. Effect of mebendazole on human cystic echinococcosis: the role of dosage and treatment duration. *Ann Trop Med Parasitol* 1999; **93**: 357-365
60 **Senyüz OF**, Yeşildag E, Celayir S. Albendazole therapy in the treatment of hydatid liver disease. *Surg Today* 2001; **31**: 487-491
61 **Nahmias J**, Goldsmith R, Soibelman M, el-On J. Three- to 7-year follow-up after albendazole treatment of 68 patients with cystic echinococcosis (hydatid disease). *Ann Trop Med Parasitol* 1994; **88**: 295-304
62 **Kumar A**, Lal BK, Chattopadhyay TK. Hydatid disease of the liver--non surgical options. *J Assoc Physicians India* 1993; **41**: 437-438
63 **Stojkovic M**, Zwahlen M, Teggi A, Vutova K, Cretu CM, Virdone R, Nicolaidou P, Cobanoglu N, Junghanss T. Treatment response of cystic echinococcosis to benzimidazoles: a systematic review. *PLoS Negl Trop Dis* 2009; **3**: e524
64 **Cobo F**, Yarnoz C, Sesma B, Fraile P, Aizcorbe M, Trujillo R, Diaz-de-Liaño A, Ciga MA. Albendazole plus praziquantel versus albendazole alone as a pre-operative treatment in intra-abdominal hydatisosis caused by Echinococcus granulosus. *Trop Med Int Health* 1998; **3**: 462-466
65 **Eckert J,** Gemmell MA, Meslin FX, Pawłowski ZS. WHO/OIE Manual on Echinococcosis in Humans and Animals: a Public Health Problem of Global Concern. World Organisation for Animal Health (Office International des Epizooties) and World Health Organization. Paris: WHO, 2001
66 **Eckert J.** Alveolar echinococcosis (Echinococcus multilocularis) and other forms of echinococcosis (Echinococcus oligarthrus and Echinococcus vogeli). In: Palmer SR, Soulsby EJL, Simpson DIH, editors. Oxford: Oxford University Press, 1998: 689-716
67 **Ricard-Blum S**, Bresson-Hadni S, Guerret S, Grenard P, Volle PJ, Risteli L, Grimaud JA, Vuitton DA. Mechanism of collagen network stabilization in human irreversible granulomatous liver fibrosis. *Gastroenterology* 1996; **111**: 172-182
68 **Grenard P**, Bresson-Hadni S, El Alaoui S, Chevallier M, Vuitton DA, Ricard-Blum S. Transglutaminase-mediated cross-linking is involved in the stabilization of extracellular matrix in human liver fibrosis. *J Hepatol* 2001; **35**: 367-375
69 **Vuitton DA**, Guerret-Stocker S, Carbillet JP, Mantion G, Miguet JP, Grimaud JA. Collagen immunotyping of the hepatic fibrosis in human alveolar echinococcosis. *Z Parasitenkd* 1986; **72**: 97-104
70 **Gottstein B**, Hemphill A. Immunopathology of echinococcosis. *Chem Immunol* 1997; **66**: 177-208
71 **Emery I**, Leclerc C, Sengphommachanh K, Vuitton DA, Liance M. In vivo treatment with recombinant IL-12 protects C57BL/6J mice against secondary alveolar echinococcosis. *Parasite Immunol* 1998; **20**: 81-91
72 **Liance M**, Ricard-Blum S, Emery I, Houin R, Vuitton DA. Echinococcus multilocularis infection in mice: in vivo treatment with a low dose of IFN-gamma decreases metacestode growth and liver fibrogenesis. *Parasite* 1998; **5**: 231-237
73 **Sturm D**, Menzel J, Gottstein B, Kern P. Interleukin-5 is the predominant cytokine produced by peripheral blood mononuclear cells in alveolar echinococcosis. *Infect Immun* 1995; **63**: 1688-1697
74 **Godot V**, Harraga S, Deschaseaux M, Bresson-Hadni S, Gottstein B, Emilie D, Vuitton DA. Increased basal production of interleukin-10 by peripheral blood mononuclear cells in human alveolar echinococcosis. *Eur Cytokine Netw* 1997; **8**: 401-408
75 **Godot V**, Harraga S, Beurton I, Deschaseaux M, Sarciron E, Gottstein B, Vuitton DA. Resistance/susceptibility to Echinococcus multilocularis infection and cytokine profile in humans. I. Comparison of patients with progressive and abortive lesions. *Clin Exp Immunol* 2000; **121**: 484-490
76 **Godot V**, Harraga S, Beurton I, Tiberghien P, Sarciron E, Gottstein B, Vuitton DA. Resistance/susceptibility to Echinococcus multilocularis infection and cytokine profile in humans. II. Influence of the HLA B8, DR3, DQ2 haplotype. *Clin Exp Immunol* 2000; **121**: 491-498
77 **Bresson-Hadni S**, Liance M, Meyer JP, Houin R, Bresson JL, Vuitton DA. Cellular immunity in experimental Echinococcus multilocularis infection. II. Sequential and comparative

phenotypic study of the periparasitic mononuclear cells in resistant and sensitive mice. *Clin Exp Immunol* 1990; **82**: 378-383
78 **Rakha NK**, Dixon JB, Carter SD, Craig PS, Jenkins P, Folkard S. Echinococcus multilocularis antigens modify accessory cell function of macrophages. *Immunology* 1991; **74**: 652-656
79 **Kizaki T**, Ishige M, Bingyan W, Day NK, Good RA, Onoé K. Generation of CD8+ suppressor T cells by protoscoleces of Echinococcus multilocularis in vitro. *Immunology* 1993; **79**: 412-417
80 **Vuitton DA**, Bresson-Hadni S, Laroche L, Kaiserlian D, Guerret-Stocker S, Bresson JL, Gillet M. Cellular immune response in Echinococcus multilocularis infection in humans. II. Natural killer cell activity and cell subpopulations in the blood and in the periparasitic granuloma of patients with alveolar echinococcosis. *Clin Exp Immunol* 1989; **78**: 67-74
81 **Frosch PM**, Frosch M, Pfister T, Schaad V, Bitter-Suermann D. Cloning and characterisation of an immunodominant major surface antigen of Echinococcus multilocularis. *Mol Biochem Parasitol* 1991; **48**: 121-130
82 **Gottstein B**. Echinococcus multilocularis: antigenic variance between different parasite isolates. *Parasitol Res* 1991; **77**: 359-361
83 **Bretagne S**, Assouline B, Vidaud D, Houin R, Vidaud M. Echinococcus multilocularis: microsatellite polymorphism in U1 snRNA genes. *Exp Parasitol* 1996; **82**: 324-328
84 **Romig T**, Dinkel A, Mackenstedt U. The present situation of echinococcosis in Europe. *Parasitol Int* 2006; **55** Suppl: S187-S191
85 **Shaikenov BS**. Distribution and ecology of Echinococcus multilocularis in Central Asia. *Parasitol Int* 2006; **55** Suppl: S213-S219
86 **Craig PS**, Deshan L, MacPherson CN, Dazhong S, Reynolds D, Barnish G, Gottstein B, Zhirong W. A large focus of alveolar echinococcosis in central China. *Lancet* 1992; **340**: 826-831
87 **Kern P**, Bardonnet K, Renner E, Auer H, Pawlowski Z, Ammann RW, Vuitton DA, Kern P. European echinococcosis registry: human alveolar echinococcosis, Europe, 1982-2000. *Emerg Infect Dis* 2003; **9**: 343-349
88 **Eckert J**, Deplazes P. Alveolar echinococcosis in humans: the current situation in Central Europe and the need for countermeasures. *Parasitol Today* 1999; **15**: 315-319
89 **Schweiger A**, Ammann RW, Candinas D, Clavien PA, Eckert J, Gottstein B, Halkic N, Muellhaupt B, Prinz BM, Reichen J, Tarr PE, Torgerson PR, Deplazes P. Human alveolar echinococcosis after fox population increase, Switzerland. *Emerg Infect Dis* 2007; **13**: 878-882
90 **Eckert J**, Conraths FJ, Tackmann K. Echinococcosis: an emerging or re-emerging zoonosis? *Int J Parasitol* 2000; **30**: 1283-1294
91 **Hildreth MB**, Sriram S, Gottstein B, Wilson M, Schantz PM. Failure to identify alveolar echinococcosis in trappers from South Dakota in spite of high prevalence of Echinococcus multilocularis in wild canids. *J Parasitol* 2000; **86**: 75-77
92 **Doi R,** Seo H, Fukuyama Y, Nakao M, Inaoka T, Kutsumi S. Epidemiology of multilocular echinococcosis in Hokkaido. A sero-epidemiological study of hunters. *Jap J Pub Health* 1987; **34**: 357-365
93 **Sato N,** Aoki S, Matsushita M, Uchino J. Clinical features. In: Uchino J, Sato N, editors. Alveolar echinococcosis of the liver. Sapporo: Hokkaido University School of Medicine, 1993: 63-68
94 **Torgerson PR**, Schweiger A, Deplazes P, Pohar M, Reichen J, Ammann RW, Tarr PE, Halkik N, Müllhaupt B. Alveolar echinococcosis: from a deadly disease to a well-controlled infection. Relative survival and economic analysis in Switzerland over the last 35 years. *J Hepatol* 2008; **49**: 72-77
95 **Bartholomot G**, Vuitton DA, Harraga S, Shi DZ, Giraudoux P, Barnish G, Wang YH, MacPherson CN, Craig PS. Combined ultrasound and serologic screening for hepatic alveolar echinococcosis in central China. *Am J Trop Med Hyg* 2002; **66**: 23-29
96 **Bresson-Hadni S**, Delabrousse E, Blagosklonov O, Bartholomot B, Koch S, Miguet JP, André Mantion G, Angèle Vuitton D. Imaging aspects and non-surgical interventional treatment in human alveolar echinococcosis. *Parasitol Int* 2006; **55** Suppl: S267-S272
97 **Reuter S**, Nüssle K, Kolokythas O, Haug U, Rieber A, Kern P, Kratzer W. Alveolar liver echinococcosis: a comparative study of three imaging techniques. *Infection* 2001; **29**: 119-125
98 **Stumpe KD**, Renner-Schneiter EC, Kuenzle AK, Grimm F, Kadry Z, Clavien PA, Deplazes P, von Schulthess GK, Muellhaupt B, Ammann RW, Renner EL. F-18-fluorodeoxyglucose (FDG) positron-emission tomography of Echinococcus multilocularis liver lesions: prospective evaluation of its value for diagnosis and follow-up during benzimidazole therapy. *Infection* 2007; **35**: 11-18
99 **Kern P**, Wen H, Sato N, Vuitton DA, Gruener B, Shao Y, Delabrousse E, Kratzer W, Bresson-Hadni S. WHO classification of alveolar echinococcosis: principles and application. *Parasitol Int* 2006; **55** Suppl: S283-S287
100 **Scheuring UJ**, Seitz HM, Wellmann A, Hartlapp JH, Tappe D, Brehm K, Spengler U, Sauerbruch T, Rockstroh JK. Long-term benzimidazole treatment of alveolar echinococcosis with hematogenic subcutaneous and bone dissemination. *Med Microbiol Immunol* 2003; **192**: 193-195
101 **Ma L**, Ito A, Liu YH, Wang XG, Yao YQ, Yu DG, Chen YT. Alveolar echinococcosis: Em2plus-ELISA and Em18-western blots for follow-up after treatment with albendazole. *Trans R Soc Trop Med Hyg* 1997; **91**: 476-478
102 **Xiao N**, Mamuti W, Yamasaki H, Sako Y, Nakao M, Nakaya K, Gottstein B, Schantz PM, Lightowlers MW, Craig PS, Ito A. Evaluation of use of recombinant Em18 and affinity-purified Em18 for serological differentiation of alveolar echinococcosis from cystic echinococcosis and other parasitic infections. *J Clin Microbiol* 2003; **41**: 3351-3353
103 **Ito A,** Ma L, Paul M, Stefaniak J, Pawlowski ZS. Evaluation of Em18-, Em16-, antigen B-western blots, Em2plus-ELISA and four other tests for differential serodiagnosis of alveolar and cystic echinococcosis patients in Poland. *Parasitol Int* 1998; **47**: 95-99
104 **Gottstein B**, Jacquier P, Bresson-Hadni S, Eckert J. Improved primary immunodiagnosis of alveolar echinococcosis in humans by an enzyme-linked immunosorbent assay using the Em2plus antigen. *J Clin Microbiol* 1993; **31**: 373-376
105 **Lanier AP**, Trujillo DE, Schantz PM, Wilson JF, Gottstein B, McMahon BJ. Comparison of serologic tests for the diagnosis and follow-up of alveolar hydatid disease. *Am J Trop Med Hyg* 1987; **37**: 609-615
106 **Rausch RL**, Wilson JF, Schantz PM, McMahon BJ. Spontaneous death of Echinococcus multilocularis: cases diagnosed serologically (by Em2 ELISA) and clinical significance. *Am J Trop Med Hyg* 1987; **36**: 576-585
107 **Ito A**, Schantz PM, Wilson JF. Em18, a new serodiagnostic marker for differentiation of active and inactive cases of alveolar hydatid disease. *Am J Trop Med Hyg* 1995; **52**: 41-44
108 **Ito A**, Xiao N, Liance M, Sato MO, Sako Y, Mamuti W, Ishikawa Y, Nakao M, Yamasaki H, Nakaya K, Bardonnet K, Bresson-Hadni S, Vuitton DA. Evaluation of an enzyme-linked immunosorbent assay (ELISA) with affinity-purified Em18 and an ELISA with recombinant Em18 for differential diagnosis of alveolar echinococcosis: results of a blind test. *J Clin Microbiol* 2002; **40**: 4161-4165
109 **Vuitton DA**, Zhang SL, Yang Y, Godot V, Beurton I, Mantion G, Bresson-Hadni S. Survival strategy of Echinococcus multilocularis in the human host. *Parasitol Int* 2006; **55** Suppl: S51-S55
110 **Wen H**, Craig PS, Ito A, Vuitton DA, Bresson-Hadni S, Allan JC, Rogan MT, Paollilo E, Shambesh M. Immunoblot evaluation of IgG and IgG-subclass antibody responses for im-

munodiagnosis of human alveolar echinococcosis. *Ann Trop Med Parasitol* 1995; **89**: 485-495
111 **Wen H**, Craig PS. Immunoglobulin G subclass responses in human cystic and alveolar echinococcosis. *Am J Trop Med Hyg* 1994; **51**: 741-748
112 **Dreweck CM**, Lüder CG, Soboslay PT, Kern P. Subclass-specific serological reactivity and IgG4-specific antigen recognition in human echinococcosis. *Trop Med Int Health* 1997; **2**: 779-787
113 **Wen H**, Bresson-Hadni S, Vuitton DA, Lenys D, Yang BM, Ding ZX, Craig PS. Analysis of immunoglobulin G subclass in the serum antibody responses of alveolar echinococcosis patients after surgical treatment and chemotherapy as an aid to assessing the outcome. *Trans R Soc Trop Med Hyg* 1995; **89**: 692-697
114 **Yamasaki H,** Nakaya K, Nakao M, Sako YAI. Significance of molecular diagnosis using histopathological specimens in cestode zoonoses. *Trop Med Health* 2007; **35**: 307-321
115 **Kadry Z**, Renner EC, Bachmann LM, Attigah N, Renner EL, Ammann RW, Clavien PA. Evaluation of treatment and long-term follow-up in patients with hepatic alveolar echinococcosis. *Br J Surg* 2005; **92**: 1110-1116
116 **Buttenschoen K**, Carli Buttenschoen D, Gruener B, Kern P, Beger HG, Henne-Bruns D, Reuter S. Long-term experience on surgical treatment of alveolar echinococcosis. *Langenbecks Arch Surg* 2009; **394**: 689-698
117 **Koch S**, Bresson-Hadni S, Miguet JP, Crumbach JP, Gillet M, Mantion GA, Heyd B, Vuitton DA, Minello A, Kurtz S. Experience of liver transplantation for incurable alveolar echinococcosis: a 45-case European collaborative report. *Transplantation* 2003; **75**: 856-863
118 **Reuter S**, Jensen B, Buttenschoen K, Kratzer W, Kern P. Benzimidazoles in the treatment of alveolar echinococcosis: a comparative study and review of the literature. *J Antimicrob Chemother* 2000; **46**: 451-456
119 **Reuter S**, Buck A, Grebe O, Nüssle-Kügele K, Kern P, Manfras BJ. Salvage treatment with amphotericin B in progressive human alveolar echinococcosis. *Antimicrob Agents Chemother* 2003; **47**: 3586-3591
120 **Kern P,** Abboud P, Kern W, Stich A, Bresson-Hadni S, Guerin B, Buttenschoen K, Gruener B, Reuter S, Hemphill A. Critical appraisal of nitazoxanide for the treatment of alveolar echinococcosis. *Am J Trop Med Hyg* 2008; **79**: 119
121 **Eckert J**, Deplazes P. Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. *Clin Microbiol Rev* 2004; **17**: 107-135

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ORIGINAL ARTICLE

Increased presence of effector lymphocytes during *Helicobacter hepaticus*-induced colitis

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Abstract

AIM: To identify and characterize drosophila mothers against decapentaplegic (SMAD)3-dependent changes in immune cell populations following infection with *Helicobacter hepaticus* (*H. hepaticus*).

METHODS: SMAD3$^{-/-}$ (n = 19) and colitis-resistant SMAD3$^{+/-}$ (n = 24) mice (8-10 wk of age) were infected with *H. hepaticus* and changes in immune cell populations [T lymphocytes, natural killer (NK) cells, T regulatory cells] were measured in the spleen and mesenteric lymph nodes (MsLNs) at 0 d, 3 d, 7 d and 28 d post-infection using flow cytometry. Genotype-dependent changes in T lymphocytes and granzyme B$^+$ cells were also assessed after 28 d in proximal colon tissue using immunohistochemistry.

RESULTS: As previously observed, SMAD3$^{-/-}$, but not SMAD3$^{+/-}$ mice, developed colitis, peaking at 4 wk post-infection. No significant changes in T cell subsets were observed in the spleen or in the MsLNs between genotypes at any time point. However, CD4$^+$ and CD8$^+$/CD62L^{lo} cells, an effector T lymphocyte population, as well as NK cells (NKp46/DX5$^+$) were significantly higher in the MsLNs of SMAD3$^{-/-}$ mice at 7 d and 28 d post-infection. In the colon, a higher number of CD3$^+$ cells were present in SMAD3$^{-/-}$ compared to SMAD3$^{+/-}$ mice at baseline, which did not significantly change during infection. However, the number of granzyme B$^+$ cells, a marker of cytolytic lymphocytes, significantly increased in SMAD3$^{-/-}$ mice 28 d post-infection compared to both SMAD3$^{+/-}$ mice and to baseline values. This was consistent with more severe colitis development in these animals.

CONCLUSION: Data suggest that defects in SMAD3 signaling increase susceptibility to *H. hepaticus*-induced colitis through aberrant activation and/or dysregulation of effector lymphocytes.

Key words: Transforming growth factor-β; Colitis; Drosophila mothers against decapentaplegic; Colon cancer; T lymphocytes

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INTRODUCTION

Individuals with inflammatory bowel disease (IBD), particularly ulcerative colitis (UC), are at a higher risk of developing colon cancer than the general population[1]. A meta-analysis of 116 studies indicated that the prevalence of colon cancer in patients with UC is approximately 3.7% (95% CI: 3.2-4.2), with the cumulative probability reaching 18% by 30 years regardless of disease severity[2]. Although the etiology of UC is poorly understood, there are indications that the immune system of individuals with UC reacts abnormally to bacteria in the digestive tract. This altered immune response leads to the inflammation-associated pathology of IBD[3-5].

Imbalances in both innate and adaptive immune cells, such as natural killer (NK) cells and T cell subsets, including $CD4^+$ and $CD8^+$ T cells and $CD4/CD25/Foxp3^+$ T regulatory (Treg) cells, are associated with the pathogenesis of IBD[2]. The inflammation and damage caused by increased secretion of inflammatory cytokines during an active disease state is thought to be triggered by cytotoxicity against the commensal bacteria[6]. For example, levels of NK cytotoxicity in UC are related to the clinical stage of the disease[7]. In active disease states, NK cells are present in normal numbers, but are functionally defective, whereas NK cells exhibit normal cytotoxic activity in an inactive disease state[7]. Induction of inflammatory cytokines can also result from the disruption of the homeostatic balance between Treg and effector T helper (Th) cells. Elevated levels of pro-inflammatory $CD4^+$ T cells lead to excess cytokine/chemokine production, thereby recruiting additional leukocytes and influencing the severity of the inflammatory response[2]. $CD8^+$ T cells are also important in the pathogenesis of UC in humans, as demonstrated by extensive $CD8^+$ T cell infiltration within intestinal lesions contributing to mucosal damage[8,9].

Transforming growth factor (TGF)-β is a multifunctional cytokine that plays an important role in epithelial and immune cell homeostasis[10,11]. TGF-β mediates many diverse biological functions on different cell types through receptor-mediated phosphorylation and activation of the drosophila mothers against decapentaplegic homolog (SMAD) family proteins, notably SMAD2 and SMAD3, which migrate to the nucleus and induce transcription of a targeted set of genes[12,13]. Dysfunctions in one or more components of TGF-β signaling are commonly observed in human IBD and during colon cancer development. For example, loss of expression of the TGF receptor type Ⅱ is observed in 90% of microsatellite instable colon cancers, leading to loss of growth regulation in epithelial cells[14]. Additionally, although the TGF-β1 isoform is overexpressed in the colon of individuals with IBD[15], nuclear signaling is impaired due to increased levels of SMAD7[16]. SMAD7 normally inhibits TGF-β signaling by blocking activation of SMAD2/3 in response to receptor-ligand binding. Normalizing SMAD7 expression restores TGF-β signaling through SMAD3 and inhibits proinflammatory cytokine production by lamina propria mononuclear cells[16].

Impairments in one or more components of the TGF-β signaling pathway are implicated in intestinal inflammation in rodent models. For example, homologous knockout of the *TGF-β1* gene in mice causes an excessive inflammatory response in multiple organs, including the heart, lungs, and intestinal tract leading to premature death[17,18]. Additionally, Maggio-Price *et al*[19] have demonstrated that disruption of the transcription factor SMAD3 modulates colitis susceptibility following infection with certain *Helicobacter spp*. Among these, *Helicobacter hepaticus* (*H. hepaticus*) is a Gram-negative spiral bacterium that colonizes the lower intestine and the hepatobiliary tract of mice[20]. Although generally asymptomatic, infection can lead to hepatic and intestinal inflammation in certain strains of immunodeficient mice[21-24]. In the complete absence of SMAD3 signaling, *H. hepaticus* induces a moderate inflammatory response in the cecum and colon, eventually leading to mucinous adenocarcinoma formation after 15-30 wk[19]. It is generally accepted that chronic low levels of inflammation lead to cancer promotion and progression[25-28], therefore, the SMAD3 mouse model is very similar to the development of specific human cancers where pathogen-induced inflammation is necessary (but not sufficient) to cause dysplasia and tumor formation.

Using this model, the focus of the current study was to investigate the effect of SMAD3 deficiency on changes in local and systemic immune cell populations following infection with *H. hepaticus*. We hypothesized that colitis susceptibility in $SMAD3^{-/-}$ mice induced by *H. hepaticus* is associated with altered immune cell populations compared to colitis resistant $SMAD3^{+/-}$ mice. The aims of this study were to: (1) characterize the colitis induced by *H. hepaticus* in colitis-sensitive $SMAD3^{-/-}$ *vs* resistant $SMAD3^{+/-}$ mice; (2) compare the immune cell population changes in the spleen and mesenteric lymph nodes (MsLNs); and (3) compare local immune cell changes by immunohistochemistry in the colon.

MATERIALS AND METHODS

Murine model

$SMAD3^{+/-}$ and $SMAD3^{-/-}$ (129-$Smad3^{tm1Par}$/J) mice were bred in-house. Homozygous males and heterozygous females were mated to obtain both $SMAD3^{+/-}$ and $SMAD3^{-/-}$ pups. Genotypes were confirmed by polymerase chain reaction (PCR). Animals were housed under specific pathogen-free (SPF) conditions in 60 square

inch plastic cages (maximum of five adult mice per cage) with microisolator lids in an Association for Assessment and Accreditation of Laboratory Animal Care-approved facility at Michigan State University. SPF conditions were assured through quarterly serology testing by Charles Rivers (Wilmington, MA, United States) and in-house testing for ectoparasites, endoparasites and fecal *Helicobacter* species (PCR). Full necropsies (including culture and sensitivity) were performed at least yearly on rodent breeding colonies. Animal rooms were maintained at 23.3 ± 2.2 ℃ with a 12-h light/dark cycle. Mice were fed Harlan Teklad 7913 rodent chow and sterile water *ad libitum*. Animal protocols were approved by the Michigan State University Institutional Animal Care and Use Committee.

Bacterial culture and infection

The wild-type *H. hepaticus* strain 3B1 (ATCC 51488) was utilized for these experiments. Isolates were aseptically streaked onto sheep blood agar and incubated at 37 ℃ for 24-48 h inside GasPak™ gas generating pouch systems (BD Diagnostic Systems, Sparks, MD, United States). Mice were infected as previously described[19]. Briefly, bacteria were collected and resuspended in tryptic soy broth at $A_{600\ nm} \geqslant 1.8$. Animals were then gavaged with 0.3 mL doses of fresh bacterial suspension on two consecutive days. Previously, Maggio-Price *et al*[19] have shown that *Helicobacter* infection is localized primarily in the cecum and proximal colon, and that bacterial DNA is still present in the tissue and luminal contents of the cecum at 12 wk post-infection. Bacterial presence was confirmed in the current study *via* DNA isolation at 3 d post-infection using a commercial kit (QIAGEN tissue kit; Valencia, CA, United States) as previously described[24].

Experimental design

In study 1, SMAD3$^{-/-}$ mice (n = 30) at 8-10 wk of age were infected with *H. hepaticus* to determine onset and duration of colitis. At the time of necropsy, mice were asphyxiated with CO_2 and exsanguinated *via* cardiac puncture. Intestinal tissue was collected and processed for histopathology at 2-8 wk post-infection. In study 2, SMAD3$^{+/-}$ (n = 24) and SMAD3$^{-/-}$ mice (n = 19) at 8-10 wk of age were infected with *H. hepaticus* once per day for two consecutive days. At select time points after infection (0, 3, 7 and 28 d), the spleen and MsLNs were collected and processed for lymphocyte isolation as described below. Colon and cecum tissue was collected, fixed, and processed for immunohistochemistry.

Histopathology

The colon and cecum were removed and flushed with phosphate-buffered saline (PBS). Tissues were fixed in 10% formalin overnight, embedded in paraffin, then sectioned and stained with hematoxylin and eosin (HE). Longitudinal sections were graded for inflammation and epithelial dysplasia/neoplasia by a pathologist using a blinded scoring system adapted from Maggio-Price *et al*[29]. Cecum and colons were scored on a 1 to 4 scale both for inflammation (1, no inflammation; 2, mild inflammation; 3, moderate inflammation; 4, marked inflammation) and dysplasia (1, no dysplasia; 2, low-grade dysplasia; 3, high-grade dysplasia; 4, high-grade dysplasia with invasion/adenocarcinoma). The two scores for colon and two scores for cecum tissue in each animal were combined such that a score of 4 indicated no inflammation or dysplasia and a score of 16 reflected maximal inflammation and neoplasia.

Immunohistochemistry was performed on paraffin-embedded colon sections. Antibodies specific for CD3 and granzyme B were purchased from Abcam (Cambridge, MA, United States). Colons were sectioned at 5 μm, mounted on coated slides, deparaffinized in xylene, and rehydrated through graded ethanol-water baths. Antigen retrieval was performed using citrate buffer (10 mmol/L, pH 6.0) and a vegetable steamer. Tissues were incubated in 3% hydrogen peroxide to block endogenous peroxidase activity and then incubated overnight at 4 ℃ in primary antibody. On the following day, tissues were washed in Tris-buffered saline containing Tween-20 (0.05%), then incubated with biotinylated secondary antibodies followed by streptavidin horseradish peroxidase for 45 min each at room temperature (Dako, Carpentaria, CA, United States). After extensive washing, antigen-bound horseradish peroxidase was detected using the chromagen 3,3'-diaminobenzidine (0.5 mg/mL; Sigma-Aldrich, St. Louis, MO, United States) dissolved in PBS (10 mmol/L, pH 7.2). Identification of cellular infiltrate in the colons of mice was performed by a pathologist. $CD3^+$ and granzyme B^+ cells were identified under a light microscope using a 20 × objective. The occurrence of positively stained cells was scored in proximal colons of mice in five fields using a 1-cm^2 grid reticle as follows: 0 = average of 0 cells/grid, 1 = average of ⩽ 1 cell/grid, 2 = average of 2-10 cells/grid, 3 = average of 11-20 cells/grid, 4 = average of > 21 cells/grid. Final values represent mean ± SE per group (n = 3-5/group).

Lymphocyte isolation

Spleens and MsLNs were removed and placed in ice-cold RPMI medium at the time of necropsy. Spleens were processed with a dounce homogenizer, pelleted, and washed in RPMI. Cells were resuspended in ACK lysing buffer (Invitrogen, Carlsbad, CA, United States) and washed twice in RPMI. MsLNs were treated with 5 mL enzymatic digest [5% fetal bovine serum (FBS), 0.5 mg/mL collagenase, 0.05 mg/mL DNase I) for 30 min at 37 ℃. Cells were passed through 70-μm filters and washed with RPMI. Cell counts were performed with a hemocytometer using trypan blue exclusion and resuspended to a concentration of one million cells per milliliter of medium.

Flow cytometry

Lymphocytes were resuspended in fluorescence-activated cell sorting (FACS) buffer (0.1% sodium azide, 1% FBS, in dPBS) blocked with anti-Fc receptor RⅡ/Ⅲ [CD16/

CD32 (purified from clone 2.4G2 hybridoma; ATCC, Manassas, VA, United States)] for 10 min on ice, and subsequently incubated with combinations of the following fluorochrome-conjugated antibodies (E-bioscience, San Diego, CA, United States; or BD Bioscience, San José, CA, United States) at concentrations ranging from 1:100 to 1:300 in FACS buffer: CD3 (PerCP-Cy5.5), CD4 (eFluor450), CD8 (PE-Cy7), CD25 (PE), FoxP3 (FITC or Alexa Fluor488), CD62 (APC), Nkp46 (FITC) and DX5 (APC). Cells were incubated in staining cocktails (one million cells per cocktail) on ice in the dark for 30 min. Intracellular staining was performed using FoxP3 staining buffer set as per the manufacturer's instructions (E-bioscience). Briefly, after surface staining, cells were washed twice in FACS buffer, fixed in 4% paraformaldehyde for 25 min, and permeabilized for 30 min. Permeabilization was followed by incubation for 30 min with the appropriate antibodies diluted in permeabilization diluent. Samples were then acquired on a LSR Ⅱ (BD Bioscience) and analyzed using FlowJo software (Tree Star Inc., Ashland, OR, United States). The number of cells in each population of interest was determined by multiplying cell percentages by the total cell number.

Statistical analysis

Data for the colitis and immunohistochemistry scores were analyzed using the nonparametric Kruskal-Wallis test and Dunn's post-test for specific comparisons. Flow cytometric data was analyzed using a two-way analysis of variance in GraphPad Prism (GraphPad Software, La Jolla, CA, United States). When statistical differences were detected, Tukey's multiple comparison test was used to determine differences between the two groups. $P < 0.05$ was considered significant. All data are represented as mean ± SE.

RESULTS

SMAD3-deficient mice are susceptible to colitis 4 wk post-infection

Colitis severity in SMAD3$^{-/-}$ mice (Figure 1A) peaked at 4 wk post-infection, with an average colitis score of 7.8 ± 0.4. This value was significant compared to samples taken at all other time points ($P < 0.05$). Colitis resolved to baseline levels in SMAD3$^{-/-}$ mice by 8 wk post-infection. In comparison, SMAD3$^{+/-}$ mice were resistant to colitis development at all time points (data not shown). There was no statistically significant change in colitis scores in SMAD3$^{+/-}$ mice compared to baseline at any time point post-infection. Representative HE images from SMAD3$^{+/-}$ and SMAD3$^{-/-}$ mice prior to and 4 wk following infection are presented in Figure 1B.

SMAD3-dependent changes in lymphocyte populations following H. hepaticus infection

We next evaluated genotype- and time-dependent changes in lymphocyte populations in the spleen and MsLNs using flow cytometry. There were no significant changes in total CD3$^+$, CD4$^+$ or CD8$^+$ lymphocytes in the spleen at baseline or at any time point following infection (Figure 2A-C). Tregs (FoxP3$^+$/CD25$^+$/CD4$^+$) and NK cells (NKp46$^+$/DX5$^+$) increased in both genotypes following infection but returned to baseline by 28 d (Figure 2D and E).

In the MsLNs, CD3$^+$, CD4$^+$ and Treg cells were significantly higher in both genotypes at 7 d and 28 d post-infection (Figure 3A, C, and D), whereas there were no significant changes in CD8$^+$ cells at any time point examined (Figure 3B). NK cells increased in SMAD3-deficient mice by 7 d post-infection, and were significantly different from baseline values at 28 d (Figure 3E). Comparably, NK cells were not significantly altered at any time point in SMAD3$^{+/-}$ mice (Figure 3E).

To determine activation status of the different T lymphocyte populations, we next evaluated surface expression of CD62L. L-Selectin (CD62L) is an adhesion marker expressed at high levels in naïve T cells and is cleaved from the surface (CD62L^{lo}) in activated and/or in memory T cells. There were no statistically significant changes or observable trends in the proportion or total number of activated T cells in the spleen at any time point after infection (data not shown). However, the proportion of CD3$^+$, CD8$^+$, CD62L^{lo} and CD3$^+$, CD4$^+$, CD62L^{lo} cells was significantly higher in SMAD3$^{-/-}$ mice at 7 d and 28 d compared to baseline values and to SMAD3$^{+/-}$ mice (Figure 4A and D). Effector Treg cells increased in both strains at 7 d and 28 d compared to baseline values (Figure 4G). CD62L expression became dimmer at later time points in the SMAD3$^{-/-}$ mice for both CD8$^+$ and CD4$^+$ populations (Figure 4C and F) in the MsLNs, however, the intensity of CD62L expression was maintained consistently in SMAD3$^{+/-}$ mice through all time points (Figure 4B and E). No significant differences were observed in the percentage of Treg cells expressing reduced levels of CD62L between genotypes at any time point (Figure 4H and I).

Immunohistochemical analysis of colon sections 28 d post-infection

We next evaluated local changes in CD3$^+$ cells and the serine protease, granzyme B, in the proximal colons of SMAD3$^{+/-}$ and SMAD3$^{-/-}$ mice 4 wk post-infection. The lamina propria in SMAD3$^{-/-}$ mice was moderately expanded by lymphocytic cells. Based on morphology and immunohistochemistry, these cells consisted primarily of CD3$^+$ lymphocytes (Figure 5A). Additionally, numerous granzyme B$^+$ cells were noted in the intestine of SMAD3$^{-/-}$ infected mice, primarily within the villous epithelium, but sometimes also within the lamina propria (Figure 5B).

DISCUSSION

Functional TGF-β signaling is crucial for maintaining immune cell homeostasis[30]. In the present study, we evaluated changes in local and systemic immune cell populations in colitis resistant SMAD3$^{+/-}$ and sensitive

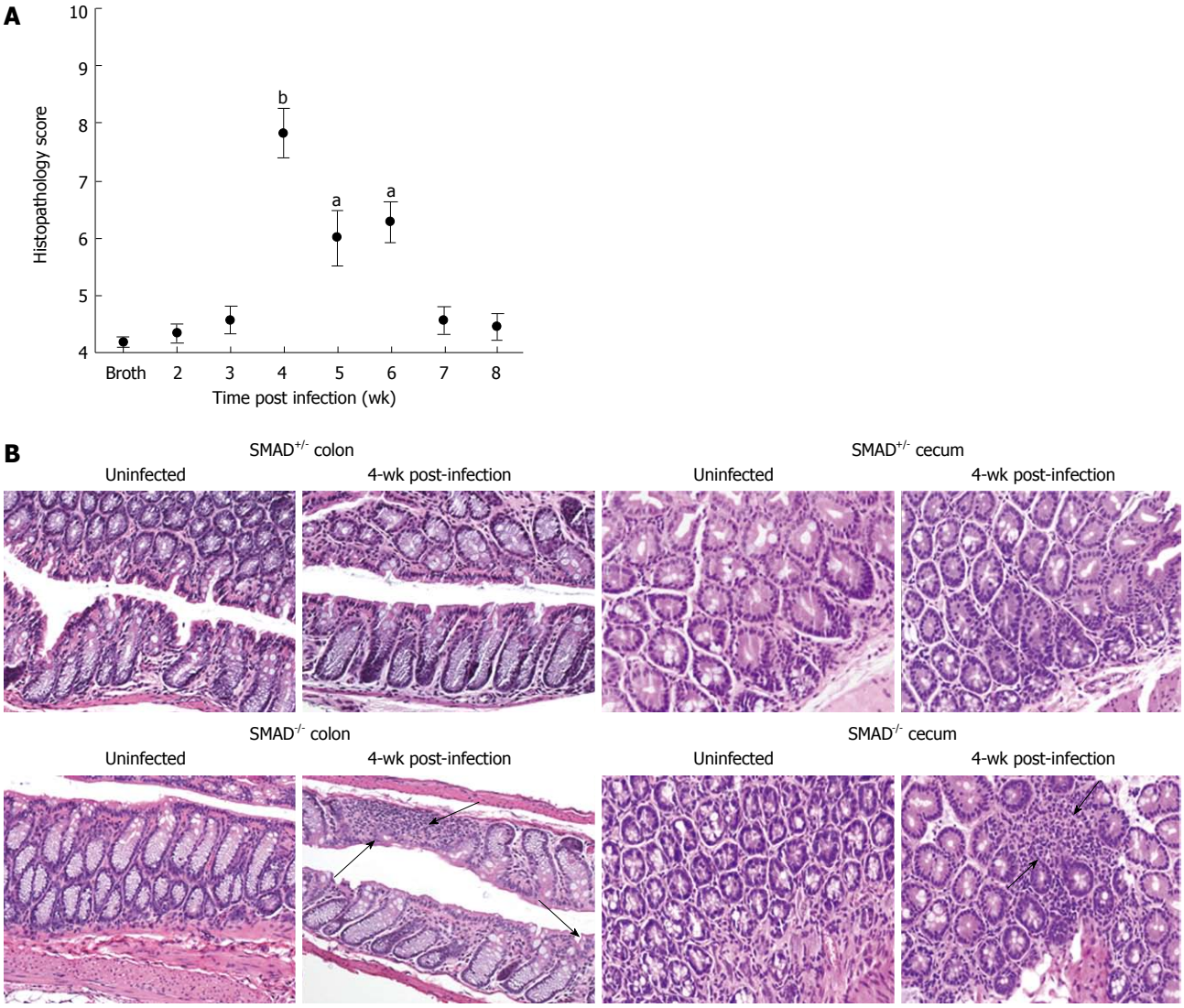

Figure 1 Drosophila mothers against decapentaplegic 3$^{+/-}$ mice are more susceptible to colitis following infection with *Helicobacter hepaticus*. A: Inflammation and dysplasia scores in drosophila mothers against decapentaplegic (SMAD)3$^{-/-}$ mice post-infection (wk). The colon and cecum from each animal were given a separate score for inflammation and dysplasia (n = 30 animals/trt). Each animal received a total of four numerical scores for each of these criteria. The figure displays the average total of these scores with a lowest possible score of 4 and a highest possible of 16. $^{a}P < 0.05$, $^{b}P < 0.001$ *vs* control animals. There was no change in colitis scores among SMAD3$^{+/-}$ mice throughout the course of infection (data not shown); B: Hematoxylin and eosin-stained sections from the cecum and colon of SMAD3$^{+/-}$ (upper panel) and SMAD3$^{-/-}$ mice (lower panel) comparing uninfected and 4 wk after infection with *Helicobacter hepaticus* (*H. hepaticus*). Four weeks following infection, the number of inflammatory cells and primarily lymphocytes in the lamina propria was slightly increased in both tissues, consistent with mild inflammation (arrows denote infiltrate).

SMAD3$^{-/-}$ mice during the course of infection with the enteric pathogen, *H. hepaticus*. A major finding of this study was a significantly higher number of CD4 and CD8 effector cell populations in the mesenteric lymph nodes of SMAD3$^{-/-}$ mice at 7 d and 28 d post-infection compared to both baseline values and to SMAD3$^{+/-}$ mice. The number of granzyme B^{+} cells, a marker of cytolytic lymphocytes, was also higher in proximal colon tissue at 28 d post-infection, consistent with colitis development in these animals. Our findings suggest loss of TGF-β signaling through SMAD3 leads to aberrant activation of colitogenic T cell subsets in response to *H. hepaticus,* whereas changes in specific T cell numbers were unaffected by genotype. These data are consistent with Maggio-Price *et al*[19], who reported no significant T cell response to infection with *Helicobacter in vitro*, although it is important to note that in that study only splenic lymphocytes were assessed, and that both *H. hepaticus* and *Helicobacter bilis* were used for infection. Additionally, Yang *et al*[31] reported no differences between SMAD3$^{-/-}$ and wild-type controls on development of T and B lymphocytes and NK cells, but found increased activated phenotype of T lymphocytes in SMAD3$^{-/-}$ mice that were resistant to TGF-β1 inhibition *in vitro*.

The inflammation associated with *H. hepaticus* infection in susceptible strains leads to a dysregulated Th1-type immune response, characterized by increased expression of interleukin (IL)-12 and interferon (IFN)-γ[19,32,33] as well as the proinflammatory cytokines IL-1α, IL-1β, IL-6 and tumor necrosis factor-α[19]. Treg cells normally function to control the inflammatory response by suppressing proliferation and activation of CD4^{+} and CD8^{+}

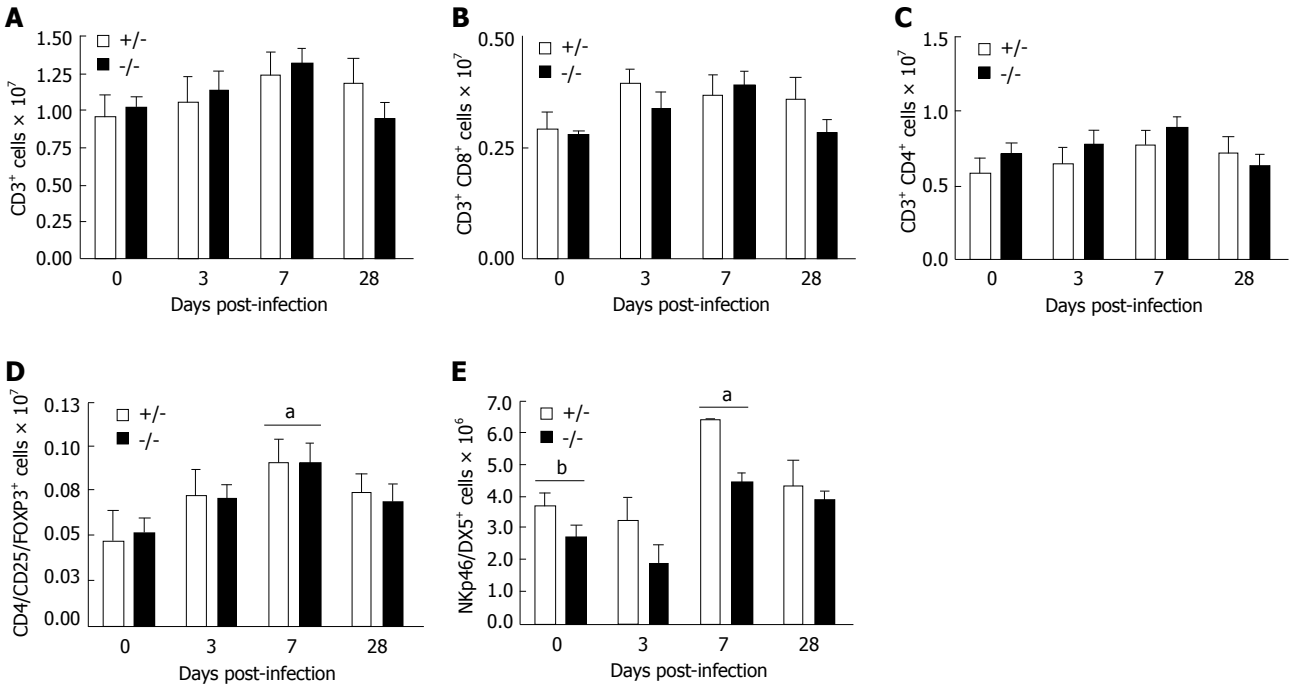

Figure 2 Changes in T lymphocyte populations and natural killer cells in the spleen of drosophila mothers against decapentaplegic 3$^{+/-}$ and drosophila mothers against decapentaplegic$^{-/-}$ mice following infection with *Helicobacter hepaticus*. Flow cytometric analysis of lymphocyte populations at days 0, 3, 7 and 28 post-infection. Gates were drawn on viable cells using forward scatter *vs* side scatter parameters. A: Total CD3$^+$ lymphocytes gated on forward scatter *vs* CD3; B: Total CD8$^+$ lymphocytes gated on CD3$^+$ lymphocytes; C: Total CD4$^+$ lymphocytes gated on CD3$^+$ lymphocytes; D: Total CD25$^+$/FOXP3$^+$ Treg cells gated on CD3$^+$/CD4$^+$ lymphocytes; E: Total natural killer (NK)p46$^+$/DX5$^+$ NK cells in spleen tissue (n = 4-6 animals per time point). $^aP < 0.05$ *vs* baseline values (7 d *vs* 0 d); $^bP < 0.05$ denotes significance between genotypes [drosophila mothers against decapentaplegic (SMAD)3$^{-/-}$ *vs* SMAD3$^{+/-}$].

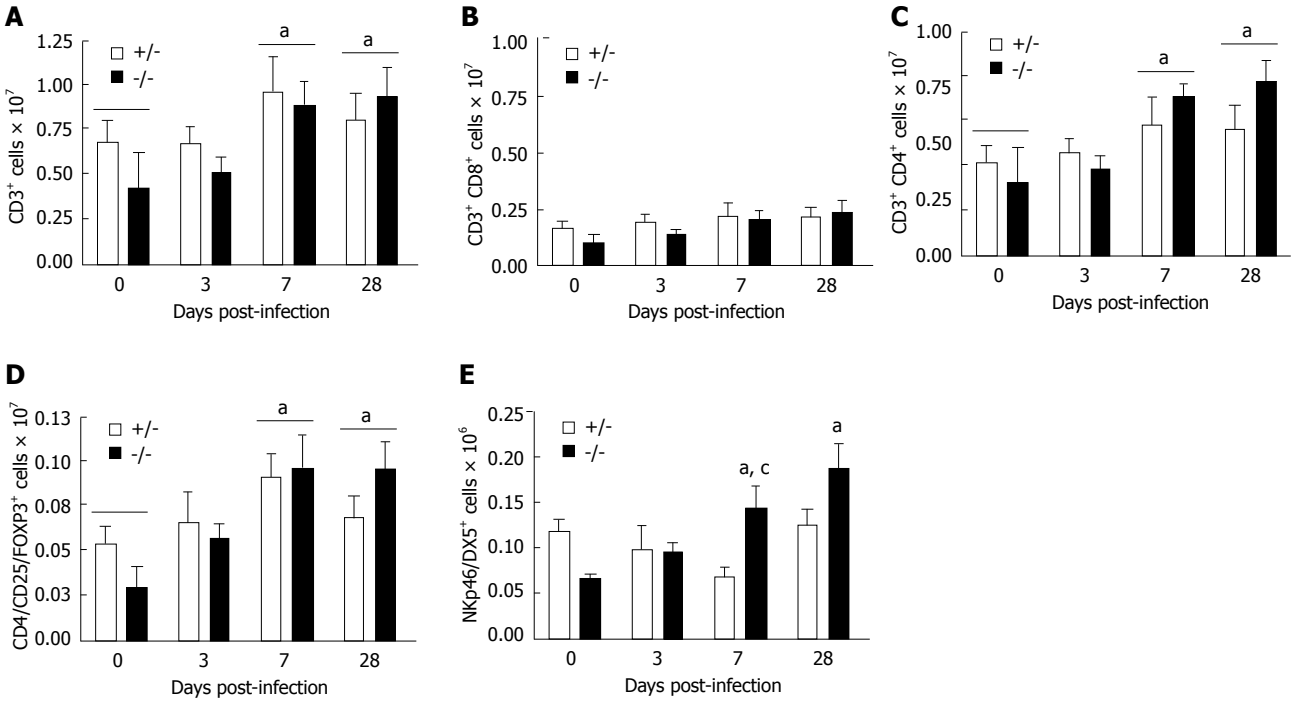

Figure 3 Changes in T lymphocyte populations and natural killer cells in the mesenteric lymph nodes of drosophila mothers against decapentaplegic 3$^{+/-}$ and drosophila mothers against decapentaplegic$^{-/-}$ mice following infection with *Helicobacter hepaticus*. Flow cytometric analysis of lymphocyte populations at days 0, 3, 7 and 28 post-infection. Gates were drawn on viable cells using forward scatter *vs* side scatter parameters. A: Total CD3$^+$ lymphocytes gated on forward scatter *vs* CD3; B: Total CD8$^+$ lymphocytes gated on CD3$^+$ lymphocytes; C: Total CD4$^+$ lymphocytes gated on CD3$^+$ lymphocytes; D: Total CD25$^+$/FOXP3$^+$ Treg cells gated on CD3$^+$/CD4$^+$ lymphocytes; E: Total natural killer (NK)p46$^+$/DX5$^+$ NK cells in mesenteric lymph nodes (n = 4-6 animals per time point). $^aP < 0.05$ *vs* baseline values; $^cP < 0.05$ denotes significant interaction between genotypes [drosophila mothers against decapentaplegic (SMAD)3$^{-/-}$ *vs* SMAD3$^{+/-}$].

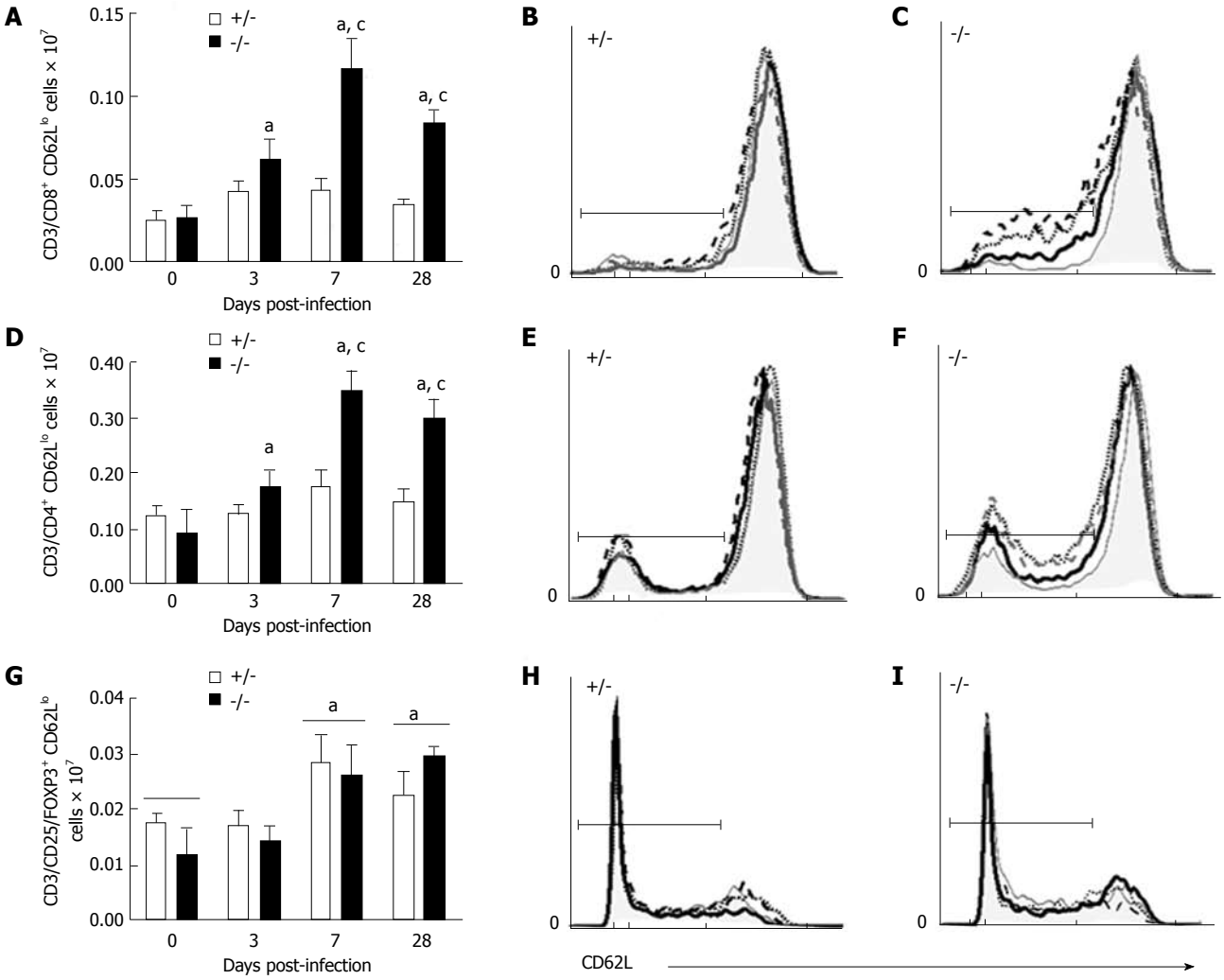

Figure 4 Changes in effector T lymphocyte populations in the mesenteric lymph nodes of drosophila mothers against decapentaplegic 3$^{+/-}$ and drosophila mothers against decapentaplegic$^{-/-}$ mice following infection with *Helicobacter hepaticus*. Flow cytometric analysis of lymphocyte populations at days 0, 3, 7 and 28 post-infection. Gates were drawn on viable cells using forward scatter *vs* side scatter parameters. Histograms represent CD62L expression at 0 d (solid grey), 3 d (dotted black), 7 d (dashed black) and 28 d (solid black) post-infection. Y-axis represents relative cell frequency. X-axis is CD62L expression. Brackets indicate CD62L^{lo} gate used to determine cell percentages. A: Total CD8$^+$ CD62L^{lo} lymphocytes gated on CD3$^+$/CD8$^+$ lymphocytes; B: CD62L expression in drosophila mothers against decapentaplegic (SMAD)3$^{+/-}$ CD8$^+$ lymphocytes; C: CD62L expression in SMAD3$^{-/-}$ CD8$^+$ lymphocytes; D: Total CD4$^+$ CD62L^{lo} lymphocytes gated on CD3$^+$/CD4$^+$ lymphocytes; E: CD62L expression in SMAD3$^{+/-}$ CD4$^+$ lymphocytes; F: CD62L expression in SMAD3$^{-/-}$ CD4$^+$ lymphocytes; G: Total CD25$^+$/FOXP3$^+$ CD62L^{lo} Treg cells gated on CD3$^+$/CD4$^+$ lymphocytes (n = 4-6 animals per time point); H: CD62L expression in SMAD3$^{+/-}$ CD25$^+$/FOXP3$^+$ lymphocytes; I: CD62L expression in SMAD3$^{-/-}$ CD25$^+$/FOXP3$^+$ lymphocytes. $^aP < 0.05$ *vs* baseline values; $^cP < 0.01$ denotes significant interaction between genotypes (SMAD3$^{-/-}$ *vs* SMAD3$^{+/-}$).

lymphocytes, inhibiting production of the cytokines IL-2 and IFN-γ, as well as producing the anti-inflammatory cytokine IL-10[34,35]. Transgenic mice lacking T and B lymphocytes, including *scid* and *rag*-2-deficient mice exhibit a more severe colitis that can be partially alleviated by adoptive transfer of IL-10-producing Treg cells[36-40]. Additionally, adoptive transfer of wild-type Treg cells into *rag2*-deficient mice inhibits *H. hepaticus*-induced colon cancer development[38,40], further establishing an important role for this cell type in suppressing inflammatory signaling.

Importantly, Treg cell development is intricately dependent on TGF-β signaling, whereas Treg cells themselves are a major source of this cytokine, deriving much of their suppressive function from TGF-β production as well as IL-10. Given the importance of this cell type in suppressing colitis in other models, we next evaluated whether SMAD3-deficiency impaired the development and/or activation of CD4$^+$/CD25$^+$/Foxp3$^+$ T regulatory cells. We found no significant difference between genotypes at baseline, suggesting normal development of this cell type in the absence of SMAD3 signaling. Following infection, Treg cells increased proportionally in both genotypes in both the spleen and MsLNs at 7 d and remained elevated in the latter at 28 d. To assess further whether activation of Treg cells may be impaired, we evaluated L-selectin (CD62L) expression, which is required for migration to sites of inflammation and is cleaved from the surface upon activation[41,42]. Although the number of activated Tregs (CD4$^+$/CD25$^+$/FOXP3$^+$CD62L^{lo}) increased at 7 d and 28 d post-infection in the MsLNs, there was no further difference between genotypes. Thus,

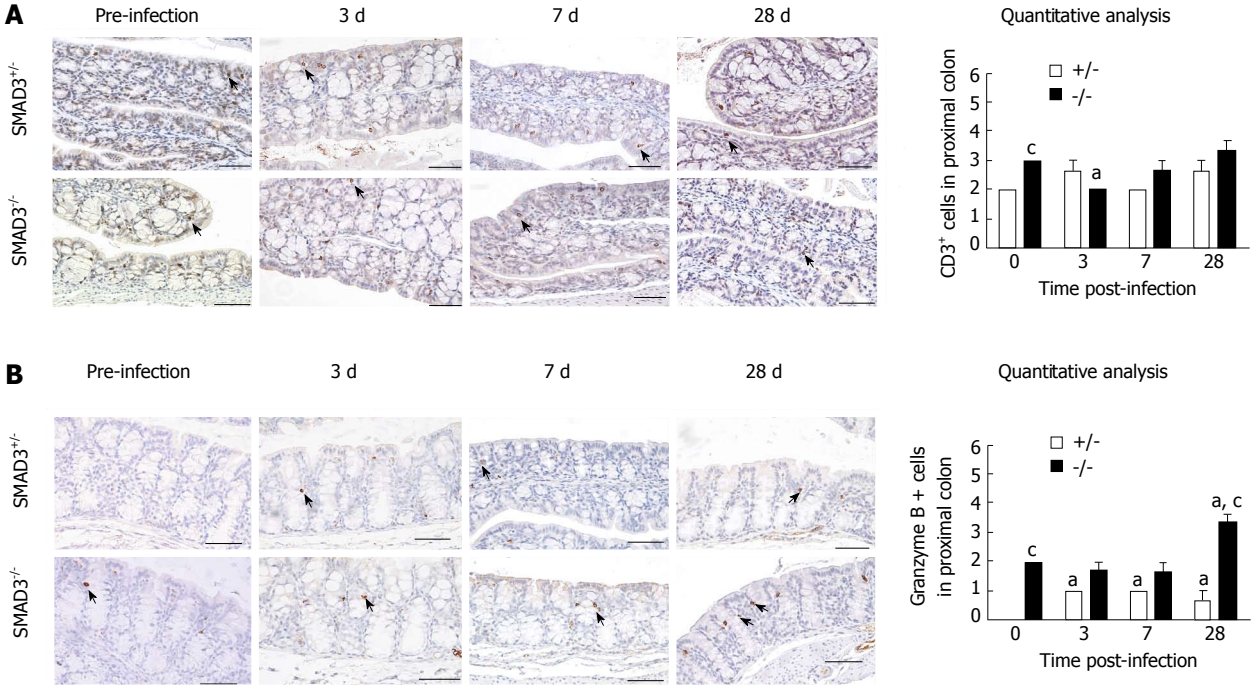

Figure 5 Immunohistochemical staining for (A) CD3 lymphocytes and (B) granzyme B in proximal colon tissue of drosophila mothers against decapentaplegic 3$^{+/-}$ and drosophila mothers against decapentaplegic$^{+/-}$ mice following infection with *Helicobacter hepaticus*. $^{a}P < 0.05$ *vs* baseline values; $^{c}P < 0.01$ denotes significant interaction between genotypes [drosophila mothers against decapentaplegic (SMAD)3$^{-/-}$ *vs* SMAD3$^{+/-}$] (n = 4-5 animals per stain). Arrows denote areas of positive staining. Scale bars represent 100 μm.

our findings suggest that SMAD3 deficiency does not influence Treg cell numbers in peripheral lymphoid tissue; however, this does not rule out the possibility that the suppressive effect of this cell type is influenced in a SMAD3-dependent manner. This is further supported by recent findings of Fantini *et al*[43] who have reported that overexpression of SMAD7 in CD4 T lymphocytes, which blocks TGF-β-mediated activation of SMAD2/3, impairs the ability of Treg cells to suppress T cell proliferation and proinflammatory cytokine expression both *in vitro* and *in vivo*.

NK cells are generally acknowledged to be important for cell-mediated immunity, and play an important role in the control of cellular infections as well as in antitumor immunity[44]. For example, NK cells can directly lyse infected/dysplastic cells through perforin-granzyme-dependent mechanisms and induce apoptosis[45-51]. Additionally, NK cells activate other effector immune cells through local production of cytokines[52]. Fort *et al*[53] have demonstrated that NK cells exert a protective effect on colitis by controlling the responses of effector CD4 T cells through perforin-dependent mechanisms[53]. Other studies have provided evidence that NK cells are in fact an innate source of IL-22 in the colon; a cytokine that has proinflammatory properties but is also proposed to protect tissues during inflammation[54-56].

Yang *et al*[31] previously have found no effect of SMAD3 deficiency on development of NK cells in the spleen or MsLNs. In the current study, we determined whether SMAD3 deficiency would influence NK cells in peripheral lymphoid tissue in response to infection with *H. hepaticus*. Surprisingly, we found higher numbers of NK cells (NKp46^{+}/DX5^{+}) in SMAD3$^{-/-}$ mice both at 7 d and 28 d post-infection in the MsLNs, whereas no corresponding changes in population numbers were observed in SMAD3$^{+/-}$ mice. Our findings of increased NK cell populations are somewhat inconsistent with the previously established protective role of this cell type[53]; however, it is possible that SMAD3 signaling mediates the balance of NK cell subsets in response to infection and/or cytotoxicity of NK cells. For example, significant enrichment of lamina propria NK cells of the CD56^{+}CD16^{+} cytotoxic subset in individuals with IBD has been reported[57]. Additionally, individuals with Crohn's disease have been reported to have a higher proportion of NKp46^{+} compared to NKp44^{+} NK cells in the intestinal mucosa, which is suggested to mediate pathogenesis through increased production of IFN-γ[58]. Importantly, TGF-β inhibits INF-γ production by NK cells[30,59], suggesting the possibility of altered balance of NK cell subsets in our model.

Our findings highlight that mice infected with *H. hepaticus* and deficient in SMAD3 signaling have elevated levels of effector lymphocyte subsets in the MsLNs and in the colon likely contributing to increased colitis severity. Although no genotype differences in numbers of natural Treg cells were found following infection, it is possible that defective TGF-β signaling through SMAD3 may impair suppressive function. Alternatively, the latent presence of effector T cells may indicate continuous antigen presenting cell stimulation which was not addressed in these studies. Given the pleiotropic role of TGF-β signaling in immune cell homeostasis, further evaluation of cytokine production by activated T cells

derived from infected SMAD3-deficient mice would lead to a more thorough understanding of SMAD3 in colitis susceptibility. Additionally, very little is known about the role of SMAD3 in NK cell function, however, the higher presence of NKp46^{+}/DX5^{+} NK cells in the MsLNs of SMAD3-deficient mice might indicate altered NK subsets present in the MsLNs due to different chemokines being released throughout the course of the infection. The signaling pathways involved in initiating the inflammatory response to *H. hepaticus* in susceptible mouse strains has also not been well characterized. *H. hepaticus* activates nuclear factor-κB and extracellular signal-regulated kinase signaling in bone-marrow-derived macrophages[60], which can induce both pro- and anti-inflammatory pathways[33,36,60,61]. Given the importance of TGF-β signaling in both IBD and colon cancer development in humans, further identifying innate targets involved in initiating the SMAD3-dependent inflammatory response to pathogenic stimuli would prove highly useful in understanding the pathogenesis of IBD as well as for designing interventions that may alter immune cell populations and/or activation. Future studies addressing some of these possibilities are currently under investigation.

ACKNOWLEDGMENTS

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COMMENTS

Background

Individuals with inflammatory bowel disease (IBD) are at an increased risk of developing colon cancer. Imbalances in immune cells, such as natural killer (NK) cells and many T cell subsets, are important in the pathogenesis of IBD. These imbalances, in addition to an abnormal reaction to natural gut bacteria, lead to increased inflammation and create an environment favorable for tumor formation in the colon.

Research frontiers

Previous studies using the drosophila mothers against decapentaplegic 3 (SMAD3) mouse model, in which SMAD3$^{-/-}$ but not SMAD3$^{+/+}$ mice develop colitis and colon cancer after infection with *Helicobacter* bacteria, highlight similarities to the development of specific human cancers in which pathogen-induced inflammation is necessary (but not sufficient) to cause dysplasia and tumor formation. This study used this model to investigate the effect of a SMAD3 deficiency on changes in both tissue-specific and systemic immune cell populations after bacterial infection.

Innovations and breakthroughs

Novel findings from this study illustrate that changes in immune response, due to genetic alteration and/or specific susceptibility, can affect the severity of colitis and potentially contribute to the development of colon tumors.

Applications

These data also suggest potential targets for prevention and treatment of chronic IBD-related inflammation. Furthermore, the SMAD3 model may also prove useful in identifying dietary and/or other interventions that alter immune cell functionality, thereby reducing inflammation and cancer.

Terminology

SMAD3 is an intracellular protein that functions as a signal transducer and transcription factor for the transforming growth factor β superfamily.

Peer review

This is an interesting manuscript. Overall, the topic is complicated and the authors present it well. There of course was a great deal of interest at one time in the treatment of ulcerative colitis with anti-*Helicobacter* antibiotics.

REFERENCES

1 **Clevers H**. At the crossroads of inflammation and cancer. *Cell* 2004; **118**: 671-674
2 **Eaden JA**, Abrams KR, Mayberry JF. The risk of colorectal cancer in ulcerative colitis: a meta-analysis. *Gut* 2001; **48**: 526-535
3 **Monteleone I**, Pallone F, Monteleone G. Interleukin-23 and Th17 cells in the control of gut inflammation. *Mediators Inflamm* 2009; **2009**: 297645
4 **Yeung MM**, Melgar S, Baranov V, Oberg A, Danielsson A, Hammarström S, Hammarström ML. Characterisation of mucosal lymphoid aggregates in ulcerative colitis: immune cell phenotype and TcR-gammadelta expression. *Gut* 2000; **47**: 215-227
5 **Tlaskalová-Hogenová H**, Tucková L, Stepánková R, Hudcovic T, Palová-Jelínková L, Kozáková H, Rossmann P, Sanchez D, Cinová J, Hrncír T, Kverka M, Frolová L, Uhlig H, Powrie F, Bland P. Involvement of innate immunity in the development of inflammatory and autoimmune diseases. *Ann N Y Acad Sci* 2005; **1051**: 787-798
6 **Baumgart DC**, Carding SR. Inflammatory bowel disease: cause and immunobiology. *Lancet* 2007; **369**: 1627-1640
7 **Manzano L**, Alvarez-Mon M, Abreu L, Antonio Vargas J, de la Morena E, Corugedo F, Durántez A. Functional impairment of natural killer cells in active ulcerative colitis: reversion of the defective natural killer activity by interleukin 2. *Gut* 1992; **33**: 246-251
8 **Ueyama H**, Kiyohara T, Sawada N, Isozaki K, Kitamura S, Kondo S, Miyagawa J, Kanayama S, Shinomura Y, Ishikawa H, Ohtani T, Nezu R, Nagata S, Matsuzawa Y. High Fas ligand expression on lymphocytes in lesions of ulcerative colitis. *Gut* 1998; **43**: 48-55
9 **Yonamine Y**, Watanabe M, Kinjo F, Hibi T. Generation of MHC class I-restricted cytotoxic T cell lines and clones against colonic epithelial cells from ulcerative colitis. *J Clin Immunol* 1999; **19**: 77-85
10 **Siegel PM**, Massagué J. Cytostatic and apoptotic actions of TGF-beta in homeostasis and cancer. *Nat Rev Cancer* 2003; **3**: 807-821
11 **Gorelik L**, Flavell RA. Transforming growth factor-beta in T-cell biology. *Nat Rev Immunol* 2002; **2**: 46-53
12 **Massagué J**. TGF-beta signal transduction. *Annu Rev Biochem* 1998; **67**: 753-791
13 **Lin Y**, Martin J, Gruendler C, Farley J, Meng X, Li BY, Lechleider R, Huff C, Kim RH, Grasser WA, Paralkar V, Wang T. A novel link between the proteasome pathway and the signal transduction pathway of the bone morphogenetic proteins (BMPs). *BMC Cell Biol* 2002; **3**: 15
14 **Grady WM**, Myeroff LL, Swinler SE, Rajput A, Thiagalingam S, Lutterbaugh JD, Neumann A, Brattain MG, Chang J, Kim SJ, Kinzler KW, Vogelstein B, Willson JK, Markowitz S. Mutational inactivation of transforming growth factor beta receptor type II in microsatellite stable colon cancers. *Cancer Res* 1999; **59**: 320-324
15 **Stadnicki A**, Machnik G, Klimacka-Nawrot E, Wolanska-Karut A, Labuzek K. Transforming growth factor-beta1 and its receptors in patients with ulcerative colitis. *Int Immunopharmacol* 2009; **9**: 761-766
16 **Monteleone G**, Kumberova A, Croft NM, McKenzie C, Steer HW, MacDonald TT. Blocking Smad7 restores TGF-beta1 signaling in chronic inflammatory bowel disease. *J Clin Invest* 2001; **108**: 601-609
17 **Kulkarni AB**, Huh CG, Becker D, Geiser A, Lyght M, Flan-

ders KC, Roberts AB, Sporn MB, Ward JM, Karlsson S. Transforming growth factor beta 1 null mutation in mice causes excessive inflammatory response and early death. *Proc Natl Acad Sci USA* 1993; **90**: 770-774
18 **Shull MM**, Ormsby I, Kier AB, Pawlowski S, Diebold RJ, Yin M, Allen R, Sidman C, Proetzel G, Calvin D. Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. *Nature* 1992; **359**: 693-699
19 **Maggio-Price L**, Treuting P, Zeng W, Tsang M, Bielefeldt-Ohmann H, Iritani BM. Helicobacter infection is required for inflammation and colon cancer in SMAD3-deficient mice. *Cancer Res* 2006; **66**: 828-838
20 **Fox JG**, Dewhirst FE, Tully JG, Paster BJ, Yan L, Taylor NS, Collins MJ, Gorelick PL, Ward JM. Helicobacter hepaticus sp. nov., a microaerophilic bacterium isolated from livers and intestinal mucosal scrapings from mice. *J Clin Microbiol* 1994; **32**: 1238-1245
21 **Foltz CJ**, Fox JG, Cahill R, Murphy JC, Yan L, Shames B, Schauer DB. Spontaneous inflammatory bowel disease in multiple mutant mouse lines: association with colonization by Helicobacter hepaticus. *Helicobacter* 1998; **3**: 69-78
22 **Fox JG**, Yan L, Shames B, Campbell J, Murphy JC, Li X. Persistent hepatitis and enterocolitis in germfree mice infected with Helicobacter hepaticus. *Infect Immun* 1996; **64**: 3673-3681
23 **Ward JM**, Anver MR, Haines DC, Melhorn JM, Gorelick P, Yan L, Fox JG. Inflammatory large bowel disease in immunodeficient mice naturally infected with Helicobacter hepaticus. *Lab Anim Sci* 1996; **46**: 15-20
24 **Pratt JS**, Sachen KL, Wood HD, Eaton KA, Young VB. Modulation of host immune responses by the cytolethal distending toxin of Helicobacter hepaticus. *Infect Immun* 2006; **74**: 4496-4504
25 **Hagemann T**, Balkwill F, Lawrence T. Inflammation and cancer: a double-edged sword. *Cancer Cell* 2007; **12**: 300-301
26 **Wilson J**, Balkwill F. The role of cytokines in the epithelial cancer microenvironment. *Semin Cancer Biol* 2002; **12**: 113-120
27 **Balkwill F**, Coussens LM. Cancer: an inflammatory link. *Nature* 2004; **431**: 405-406
28 **Balkwill F**, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet* 2001; **357**: 539-545
29 **Maggio-Price L**, Bielefeldt-Ohmann H, Treuting P, Iritani BM, Zeng W, Nicks A, Tsang M, Shows D, Morrissey P, Viney JL. Dual infection with Helicobacter bilis and Helicobacter hepaticus in p-glycoprotein-deficient mdr1a-/- mice results in colitis that progresses to dysplasia. *Am J Pathol* 2005; **166**: 1793-1806
30 **Kriegel MA**, Li MO, Sanjabi S, Wan YY, Flavell RA. Transforming growth factor-beta: recent advances on its role in immune tolerance. *Curr Rheumatol Rep* 2006; **8**: 138-144
31 **Yang X**, Letterio JJ, Lechleider RJ, Chen L, Hayman R, Gu H, Roberts AB, Deng C. Targeted disruption of SMAD3 results in impaired mucosal immunity and diminished T cell responsiveness to TGF-beta. *EMBO J* 1999; **18**: 1280-1291
32 **Kullberg MC**, Ward JM, Gorelick PL, Caspar P, Hieny S, Cheever A, Jankovic D, Sher A. Helicobacter hepaticus triggers colitis in specific-pathogen-free interleukin-10 (IL-10)-deficient mice through an IL-12- and gamma interferon-dependent mechanism. *Infect Immun* 1998; **66**: 5157-5166
33 **Tomczak MF**, Erdman SE, Poutahidis T, Rogers AB, Holcombe H, Plank B, Fox JG, Horwitz BH. NF-kappa B is required within the innate immune system to inhibit microflora-induced colitis and expression of IL-12 p40. *J Immunol* 2003; **171**: 1484-1492
34 **Thornton AM**, Shevach EM. CD4+CD25+ immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting interleukin 2 production. *J Exp Med* 1998; **188**: 287-296
35 **Piccirillo CA**, Shevach EM. Cutting edge: control of CD8+ T cell activation by CD4+CD25+ immunoregulatory cells. *J Immunol* 2001; **167**: 1137-1140
36 **Tomczak MF**, Erdman SE, Davidson A, Wang YY, Nambiar PR, Rogers AB, Rickman B, Luchetti D, Fox JG, Horwitz BH. Inhibition of Helicobacter hepaticus-induced colitis by IL-10 requires the p50/p105 subunit of NF-kappa B. *J Immunol* 2006; **177**: 7332-7339
37 **von Freeden-Jeffry U**, Davidson N, Wiler R, Fort M, Burdach S, Murray R. IL-7 deficiency prevents development of a non-T cell non-B cell-mediated colitis. *J Immunol* 1998; **161**: 5673-5680
38 **Erdman SE**, Rao VP, Poutahidis T, Ihrig MM, Ge Z, Feng Y, Tomczak M, Rogers AB, Horwitz BH, Fox JG. CD4(+)CD25(+) regulatory lymphocytes require interleukin 10 to interrupt colon carcinogenesis in mice. *Cancer Res* 2003; **63**: 6042-6050
39 **Maloy KJ**, Salaun L, Cahill R, Dougan G, Saunders NJ, Powrie F. CD4+CD25+ T(R) cells suppress innate immune pathology through cytokine-dependent mechanisms. *J Exp Med* 2003; **197**: 111-119
40 **Erdman SE**, Poutahidis T, Tomczak M, Rogers AB, Cormier K, Plank B, Horwitz BH, Fox JG. CD4+ CD25+ regulatory T lymphocytes inhibit microbially induced colon cancer in Rag2-deficient mice. *Am J Pathol* 2003; **162**: 691-702
41 **Venturi GM**, Conway RM, Steeber DA, Tedder TF. CD25+-CD4+ regulatory T cell migration requires L-selectin expression: L-selectin transcriptional regulation balances constitutive receptor turnover. *J Immunol* 2007; **178**: 291-300
42 **Grailer JJ**, Kodera M, Steeber DA. L-selectin: role in regulating homeostasis and cutaneous inflammation. *J Dermatol Sci* 2009; **56**: 141-147
43 **Fantini MC**, Rizzo A, Fina D, Caruso R, Sarra M, Stolfi C, Becker C, Macdonald TT, Pallone F, Neurath MF, Monteleone G. Smad7 controls resistance of colitogenic T cells to regulatory T cell-mediated suppression. *Gastroenterology* 2009; **136**: 1308-1316, 1308-1316
44 **Lünemann A**, Lünemann JD, Münz C. Regulatory NK-cell functions in inflammation and autoimmunity. *Mol Med* 2009; **15**: 352-358
45 **Byrne P**, McGuirk P, Todryk S, Mills KH. Depletion of NK cells results in disseminating lethal infection with Bordetella pertussis associated with a reduction of antigen-specific Th1 and enhancement of Th2, but not Tr1 cells. *Eur J Immunol* 2004; **34**: 2579-2588
46 **Dunn PL**, North RJ. Early gamma interferon production by natural killer cells is important in defense against murine listeriosis. *Infect Immun* 1991; **59**: 2892-2900
47 **Harty JT**, Bevan MJ. Specific immunity to Listeria monocytogenes in the absence of IFN gamma. *Immunity* 1995; **3**: 109-117
48 **Huang S**, Hendriks W, Althage A, Hemmi S, Bluethmann H, Kamijo R, Vilcek J, Zinkernagel RM, Aguet M. Immune response in mice that lack the interferon-gamma receptor. *Science* 1993; **259**: 1742-1745
49 **Le-Barillec K**, Magalhaes JG, Corcuff E, Thuizat A, Sansonetti PJ, Phalipon A, Di Santo JP. Roles for T and NK cells in the innate immune response to Shigella flexneri. *J Immunol* 2005; **175**: 1735-1740
50 **Spörri R**, Joller N, Albers U, Hilbi H, Oxenius A. MyD88-dependent IFN-gamma production by NK cells is key for control of Legionella pneumophila infection. *J Immunol* 2006; **176**: 6162-6171
51 **Way SS**, Borczuk AC, Dominitz R, Goldberg MB. An essential role for gamma interferon in innate resistance to Shigella flexneri infection. *Infect Immun* 1998; **66**: 1342-1348
52 **Lanier LL**. NK cell recognition. *Annu Rev Immunol* 2005; **23**: 225-274
53 **Fort MM**, Leach MW, Rennick DM. A role for NK cells as regulators of CD4+ T cells in a transfer model of colitis. *J Immunol* 1998; **161**: 3256-3261
54 **Zenewicz LA**, Yancopoulos GD, Valenzuela DM, Murphy

AJ, Stevens S, Flavell RA. Innate and adaptive interleukin-22 protects mice from inflammatory bowel disease. *Immunity* 2008; **29**: 947-957

55 **Radaeva S**, Sun R, Pan HN, Hong F, Gao B. Interleukin 22 (IL-22) plays a protective role in T cell-mediated murine hepatitis: IL-22 is a survival factor for hepatocytes via STAT3 activation. *Hepatology* 2004; **39**: 1332-1342

56 **Zenewicz LA**, Yancopoulos GD, Valenzuela DM, Murphy AJ, Karow M, Flavell RA. Interleukin-22 but not interleukin-17 provides protection to hepatocytes during acute liver inflammation. *Immunity* 2007; **27**: 647-659

57 **Steel AW**, Mela CM, Lindsay JO, Gazzard BG, Goodier MR. Increased proportion of CD16(+) NK cells in the colonic lamina propria of inflammatory bowel disease patients, but not after azathioprine treatment. *Aliment Pharmacol Ther* 2011; **33**: 115-126

58 **Takayama T**, Kamada N, Chinen H, Okamoto S, Kitazume MT, Chang J, Matuzaki Y, Suzuki S, Sugita A, Koganei K, Hisamatsu T, Kanai T, Hibi T. Imbalance of NKp44(+)NKp46(-) and NKp44(-)NKp46(+) natural killer cells in the intestinal mucosa of patients with Crohn's disease. *Gastroenterology* 2010; **139**: 882-892, 892.e1-3

59 **Laouar Y**, Sutterwala FS, Gorelik L, Flavell RA. Transforming growth factor-beta controls T helper type 1 cell development through regulation of natural killer cell interferon-gamma. *Nat Immunol* 2005; **6**: 600-607

60 **Tomczak MF**, Gadjeva M, Wang YY, Brown K, Maroulakou I, Tsichlis PN, Erdman SE, Fox JG, Horwitz BH. Defective activation of ERK in macrophages lacking the p50/p105 subunit of NF-kappaB is responsible for elevated expression of IL-12 p40 observed after challenge with Helicobacter hepaticus. *J Immunol* 2006; **176**: 1244-1251

61 **Wang Y**, Rickman BH, Poutahidis T, Schlieper K, Jackson EA, Erdman SE, Fox JG, Horwitz BH. c-Rel is essential for the development of innate and T cell-induced colitis. *J Immunol* 2008; **180**: 8118-8125

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ORIGINAL ARTICLE

Immunological milieu in the peritoneal cavity at laparotomy for gastric cancer

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Abstract

AIM: To investigate the immunological repertoire in the peritoneal cavity of gastric cancer patients.

METHODS: The peritoneal cavity is a compartment in which immunological host-tumor interactions can occur. However, the role of lymphocytes in the peritoneal cavity of gastric cancer patients is unclear. We observed 64 patients who underwent gastrectomy for gastric cancer and 11 patients who underwent laparoscopic cholecystectomy for gallstones and acted as controls. Lymphocytes isolated from both peripheral blood and peritoneal lavage were analyzed for surface markers of lymphocytes and their cytokine production by flow cytometry. $CD4^+CD25^{high}$ T cells isolated from the patient's peripheral blood were co-cultivated for 4 d with the intra-peritoneal lymphocytes, and a cytokine assay was performed.

RESULTS: At gastrectomy, $CCR7^-$ $CD45RA^-$ $CD8^+$ effector memory T cells were observed in the peritoneal cavity. The frequency of $CD4^+$ $CD25^{high}$ T cells in both the peripheral blood and peritoneal cavity was elevated in patients at advanced stage [control *vs* stage Ⅳ in the peripheral blood: 6.89 (3.39-10.4) *vs* 15.34 (11.37-19.31), $P < 0.05$, control *vs* stage Ⅳ in the peritoneal cavity: 8.65 (5.28-12.0) *vs* 19.56 (14.81-24.32), $P < 0.05$]. On the other hand, the suppression was restored with $CD4^+$ $CD25^{high}$ T cells from their own peripheral blood. This study is the first to analyze lymphocyte and cytokine production in the peritoneal cavity in patients with gastric cancer. Immune regulation at advanced stage is reversible at the point of gastrectomy.

CONCLUSION: The immunological milieu in the peritoneal cavity of patients with advanced gastric cancer elicited a Th2 response even at gastrectomy, but this response was reversible.

Key words: Cytokines; Gastric cancer; Lymphocytes; Peritoneal cavity; Regulatory T cell

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INTRODUCTION

Tumor progression is governed not only by the genetic changes intrinsic to cancer cells, but also by epigenetic and environmental factors. Therefore, neoplastic cell factors and biophylactic side factors such as immune reactions are interacting in the survival and development of micrometastasis. Increasing evidence gleaned from studies in immune-compromised hosts suggests that the cellular mechanisms of immunosurveillance influence tumor development. There are several lines of research which indicate the critical role of the immune system in controlling the growth of malignant cells[1-5]. Thus, impairment of anti-tumor immunity, which leads to immunologic toleration of malignant cells, contributes to the development and progression of peritoneal metastasis[6]. The elimination phase of the cancer immunosurveillance mechanism is thought to be a continuous process, and local control of metastatic invasion by the immune system may be critical for survival. However, the role of lymphocytes in the peritoneal cavity for anti-tumor immunity in gastric cancer patients is unknown[7].

Studies in rodents have demonstrated that adoptive immunotherapy with antigen-specific $CD8^+$ T cells is effective for cancer, and there is evidence that this approach has therapeutic activity in humans[8-10]. Memory T cells circulate throughout all tissues of the body and are primed to rapidly produce secondary immune responses upon antigen challenge[11]. The nature of the cells that mediate the different facts of immunological memory remains unresolved. Natural killer T cells are a specialized subset of T cells. They express T-cell and natural killer-lineage cell surface markers and key cytokines, which regulate the course of the immune response. There are many mechanisms that regulate and dampen the immune response to cancers[12-15]. Regulatory T cells protect the host from autoimmune disease by suppressing self-reactive immune cells. As such, regulatory T cells may also block antitumor immune responses. Regulatory T cells have been an active research area in basic as well as in clinical immunology[16-18]. Th1 immune responses are considered to be essential for eradicating malignant cells. Based on the cytokine profile, interferon-gamma is a Th1 cytokine with an antitumor effect. Interleukin-10, a Th2 cytokine, inhibits Th1 immune responses and enhances the production of other Th2 cytokines[19-22].

In order to clarify the clinical significance of the host immune response within the peritoneal cavity in patients with gastric cancer, we conducted an immunological analysis of the peritoneal lavage obtained from patients at the time of gastrectomy.

MATERIALS AND METHODS

Patients

A total of 75 patients (50 males and 25 females; mean age: 64.3 years) were included in this study. Sixty-four patients were histologically diagnosed as having gastric cancer. Among these, 56 had gastrectomy, 2 underwent bypass operation, and 6 had exploratory laparotomy. Eleven patients who underwent laparoscopic cholecystectomy for benign disease acted as controls. The resected specimens were histologically examined by hematoxylin and eosin staining according to the general rules of the Japanese Classification of Gastric Carcinoma[23]. The investigation protocol was approved by the Institutional Review Board of the Nagasaki University School of Medicine (#14122694). Written informed consent was obtained from all patients. The stages of gastric cancer patients were as follows: stage I A, $n = 25$ patients; stage I B, $n = 13$; stage II, $n = 7$; stage III, $n = 7$; and stage IV, $n = 12$. The clinicopathological features of the patients are shown in Table 1.

Table 1 Clinicopathological features in the examined gastric cancer patients

Variables	No. of patients
Total cases	64
Age (yr)	67.5 ± 2.8
Sex (male/female)	42/22
Depth of tumor invasion	
T1	32
T2	20
T3	9
T4	3
Lymphnode metastasis	
N0	34
N1	12
N2	14
N3	4
Peritoneal metastasis	
Absent	56
Present	8
Cytology	
Negative	57
Positive	7
Stage	
Stage I A	25
Stage I B	13
Stage II	7
Stage III	7
Stage IV	12

Isolation of mononuclear cells from peripheral blood and peritoneal lavage

Endotracheal general anesthesia was induced and 10 mL of peripheral blood was taken from all patients. Four hundred milliliter of physiological saline was poured into the peritoneal cavity prior to manipulation of the tumor, and was recovered after being gently stirred. Half of the peritoneal lavage was allocated for conventional cytology and carcinoembryonic antigen (CEA) analysis by an enzyme-linked immunosorbent assay. The other half of the peritoneal lavage was immediately centrifuged at 2000 rpm for 10 min, and the supernatants were assayed for CEA values. The peritoneal CEA levels were then measured using an enzyme immunoassay kit (IMx-SERECT CEA, Dainabot, Tokyo) and the protein concentration was determined using a protein assay kit (Bio-Rad, Richmond, CA, United States). The cell component was used for lymphocyte analysis. Lymphocytes from peripheral

Table 2 Carcinoembryonic antigen values in sera and peritoneal lavage

Source	Control	Stage Ⅰ A	Stage Ⅰ B	Stage Ⅱ	Stage Ⅲ	Stage Ⅳ
CEA						
PB (ng/mL)	Not tested	2.09 (1.39-2.78)	2.03 (0.96-3.1)	3.06 (2.04-4.07)	2.54 (0.38-4.69)	7.98 (1.18-15.82)
PL (ng/g protein)	56.53 (21.82-91.24)	44.17 (27.37-60.96)	61.95 (11.98-111.91)	83.14 (7.31-187.54)	262.63 (7.26-517.26)	1234.00 (87.77-2380.22)
CD4/CD8						
PB (ratio)	5.379 (2.705-8.052)	5.595 (3.224-7.967)	4.571 (2.057-7.086)	5.277 (1.369-9.184)	7.999 (3.366-12.632)	4.156 (2.228-6.083)
PL (ratio)	0.494 (0.338-0.649)	0.553 (0.421-0.685)	0.697 (0.511-0.883)	0.638 (0.395-0.881)	1.242 (0.961-1.522)	1.158 (0.907-1.408)
CD45RA-/CCR7-						
PB (%)	60.43 (46.42-74.44)	58.29 (48.93-67.64)	53.92 (32.65-75.2)	57.36 (42.01-72.71)	49.01 (29.31-68.71)	45.73 (32.79-58.67)
PL (%)	81.17 (81.12-93.22)	81.67 (76.35-87.01)	76.2 (59.43-92.96)	72.3 (61.01-83.58)	68.36 (58.70-78.02)	51.92 (38.34-65.50)
NKT						
PB (%)	9.19 (5.83-12.54)	7.59 (5.63-9.56)	9.47 (4.41-14.53)	10.71 (1.55-19.87)	5.43 (0.54-10.33)	7.16 (3.95-10.3)
PL (%)	18.1 (9.83-26.37)	17.25 (13.54-20.97)	15.74 (9.23-22.25)	15.38 (7.71-23.04)	9.66 (1.2-18.11)	9.91 (6.94-12.88)

PB: Peripheral blood; PL: Peritoneal lavage; CI: Confidence interval. The data are presented as the median and 95% CI. The statistical analysis of the differences revealed higher CEA and CD4/CD8, lower $CD8^+$ effector memory T cells and NKT cells in the peritoneal cavity in patients with advanced stage than in controls.

blood were isolated by density centrifugation over Ficoll-Paque™ gradients (Amersham, Uppsala, Sweden).

Flow cytometry

The following monoclonal antibodies were used in the present study: fluorescein isothiocyanate (FITC)-conjugated anti-CD8, FITC-CD25, FITC-CD45RA, phycoerythrin (PE)-conjugated anti-CD4, PE-CD56, PE-CCR7, PE-IFN-γ, PE-IL-10, PE-Foxp3, cychrome (Cy)-conjugated anti-CD3, and Cy-CD8 (BD Pharmingen, San Diego, CA, United States). Single-cell suspensions were stained in phosphate-buffered saline-1% fetal calf serum at saturating concentrations according to standard procedures. Flow cytometry was performed on the BD Biosystems-FACSCanto Ⅱ system (BD Biosciences, San Diego, CA, United States), and FACSDiva software (BD Biosciences, San Diego, CA, United States) was used for analysis. All analyses of T cells were carried out after gating by CD3. The ratio of the percentage of CD4 and CD8 cells was represented as the CD4/CD8 ratio.

Intracellular staining for Foxp3

Intracellular staining for Foxp3 was performed using the Human Foxp3 Buffer set (BD Pharmingen, San Diego, CA, United States) according to the manufacturer's protocol.

Cytokine assays

Anti-IFN-γ-PE and anti-IL-10-PE mAbs were used for the intracellular analysis of cytokine production. Peripheral and intra-peritoneal lymphocytes were activated with 10 ng/mL phorbol 12-myristate-13-acetate (PMA), 0.5 μg/mL Ionomycin, and 1 μL/mL GolgiPlug (BD Pharmingen, San Diego, CA, United States) for 4 h. Cells were washed, fixed and permeabilized by Cytofix/Cytoperm solution (BD Pharmingen, San Diego, CA, United States), and stained with titrated amounts of cytokine-specific antibodies.

Next, the $CD4^+$ $CD25^+$ T cells were isolated from peripheral blood by magnetic beads (Miltenyi Biotech,BergischGladbach, Germany). These $CD4^+$ $CD25^+$ T cells were mixed with intraperitoneal lymphocytes at a ratio of 1:10 and co-cultivated for 4 d in RPMI with 10% FBS. The $CD4^+$ $CD25^-$ T cells were co-cultivated with intraperitoneal lymphocytes as controls. The cytokine assay was performed by the intracellular cytokine method after 4 d of co-cultivation.

Statistical analysis

The statistical analysis was performed using the Kruskal-Wallis test (non-parametric ANOVA) using a personal computer and the StatViewV.5.0 software package (SAS Institute, Cary, NC, United States). *P* values less than 0.05 were considered to indicate statistical significance.

RESULTS

Carcinoembryonic antigen values in sera and peritoneal lavage

For the interaction between peripheral blood and the peritoneal cavity, we investigated the CEA values in both serum and peritoneal lavage at the time of surgery. The serum CEA values were elevated only in patients with stage Ⅳ disease. On the other hand, the values in peritoneal lavage were found to be elevated even at stage Ⅲ, and they were also related to the clinical stage (Table 2).

Analysis of lymphocyte populations in peripheral blood and the peritoneal cavity

After purification of lymphocytes from peritoneal lavage, we investigated the phenotypes of lymphocytes in both peripheral blood and the peritoneal cavity. The mean value of the CD4/CD8 ratio for all patients was 2.17 in peripheral blood. The $CD8^+$ T cells were dominant in the peritoneal cavity and the CD4/CD8 ratio was reversed. The ratio in patients with stage Ⅲ or Ⅳ was significantly higher than in stage Ⅰ or control patients (Table 2).

The $CCR7^-$ $CD45RA^-$ $CD8^+$ T cells were counted as effector memory T cell subsets. The percentage of effector memory T cells in the peritoneal cavity was higher than that in peripheral blood. However, the percentage

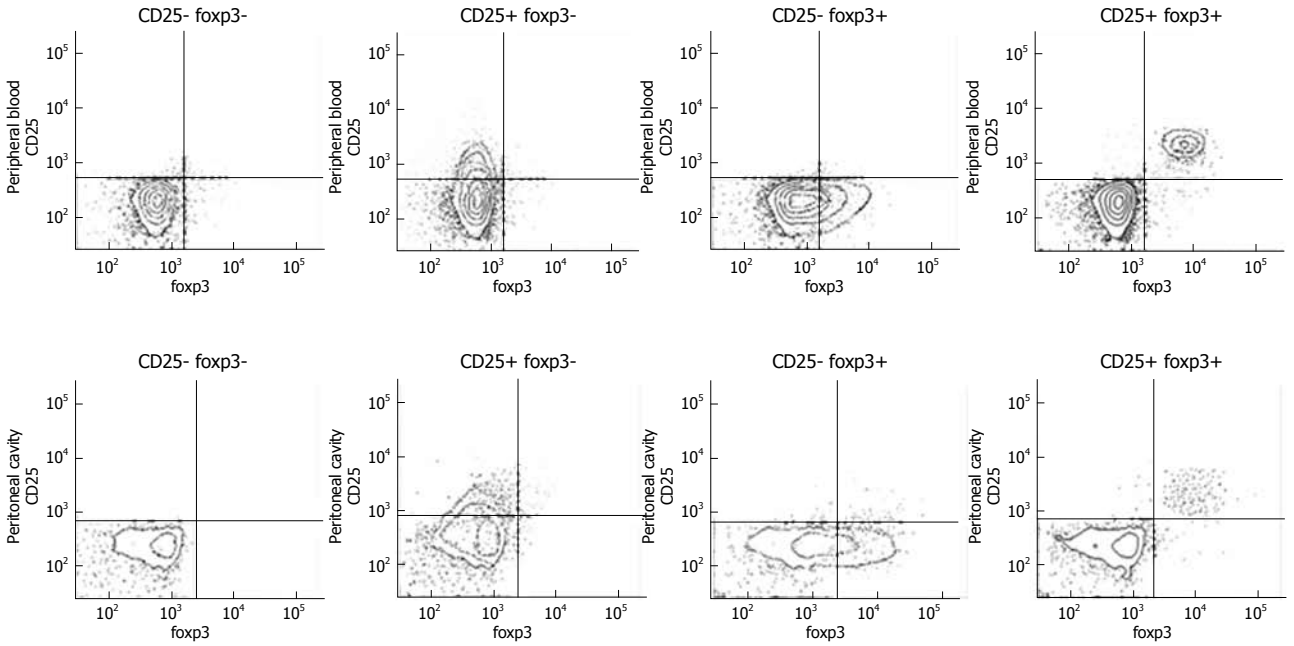

Figure 1 Co-staining with foxp3 and CD25 for CD4$^+$ T cells. High correlation was shown between both populations.

decreased in association with the clinical stage (Table 2). The CD3$^+$CD56$^+$ cells were measured as natural killer T cells. The percentage of these cells in the peritoneal lavage was also low in patients with stage Ⅲ or stage Ⅳ (Table 2). As the co-staining of foxp3 and CD25 revealed a high correlation between both populations, CD25^{high} was used following cytokine producing assays (Figure 1). The frequency of CD4$^+$ CD25^{high} T cells in patients with advanced stage cancer was higher than that in control patients in both peripheral blood and the peritoneal cavity (Figure 2A and B).

Cytokine production by lymphocytes

The cytokine production from CD3$^+$ T cells after stimulation with PMA + ionomycin was evaluated by a cytokine production assay. The lymphocytes in the peritoneal cavity were more sensitive for the production of IFN-γ than those in the peripheral blood. The ratio of IFN-γ producing cells in the peritoneal cavity was significantly lower in patients with advanced stage disease in comparison to the controls (Figure 3A and B). The ratio of IL-10 producing cells in the peritoneal cavity in patients with advanced stages was higher in comparison to the controls (Figure 3C and D).

Cytokine assays of intra-peritoneal lymphocytes after co-cultivation with self- CD4$^+$CD25^{high} T cells

In order to investigate whether the suppression of IFN-γ production from T cells in the peritoneal cavity at advanced stages was caused by CD4$^+$ CD25^{high} T cells, further assays were performed. The IFN-γ production of CD8$^+$ T cells was suppressed in intra-peritoneal lymphocytes co-cultivated with isolated CD4$^+$ CD25^{high} T cells from self-peripheral blood (Figure 4A). No inhibition was seen when the lymphocytes were co-cultivated with CD4$^+$ CD25$^-$ cells (Figure 4B).

DISCUSSION

The peritoneal cavity is a compartment in which the immunological host-tumor interaction can occur[24]. This study investigated lymphocytes in the peritoneal cavity of patients with gastric cancer in relation to anti-tumor immunity. Some tumors can acquire the ability to down-regulate immune responses and exploit this action to promote tumor cell proliferation, survival, and invasion[10,25]. Therefore, the presence of leukocytes in the peritoneal cavity may be a consequence of an immune response that favors either dissemination of tumor cells or a protective host response. Malignant ascites has been used as a common source of immunological analysis in previous reports[11,26]. To the best of our knowledge, there are no reports describing the lymphocyte and cytokine production ability in peritoneal lavage from patients with gastric cancer at the time of gastrectomy.

In our initial experiments, the CEA values in peritoneal lavage were found to correlate with the clinical stages. Interestingly, the CEA values were elevated even in cases without serosal invasion. This result suggests that some fragments of cancer cells may spread throughout the peritoneal cavity and induce an immune reaction between the tumor and host[26,27].

The frequency of CD4$^+$ T cells in all patients was higher than that of CD8$^+$ T cells in peripheral blood, but this pattern was reversed in peritoneal lavage fluid. CD8$^+$ T cells were dominant in the peritoneal cavity. Our data suggested that the immunological environment in the peripheral blood is different from that in the peritoneal cavity. There were significant differences in the CD4/CD8 ratio in the peritoneal cavity between gastric cancer

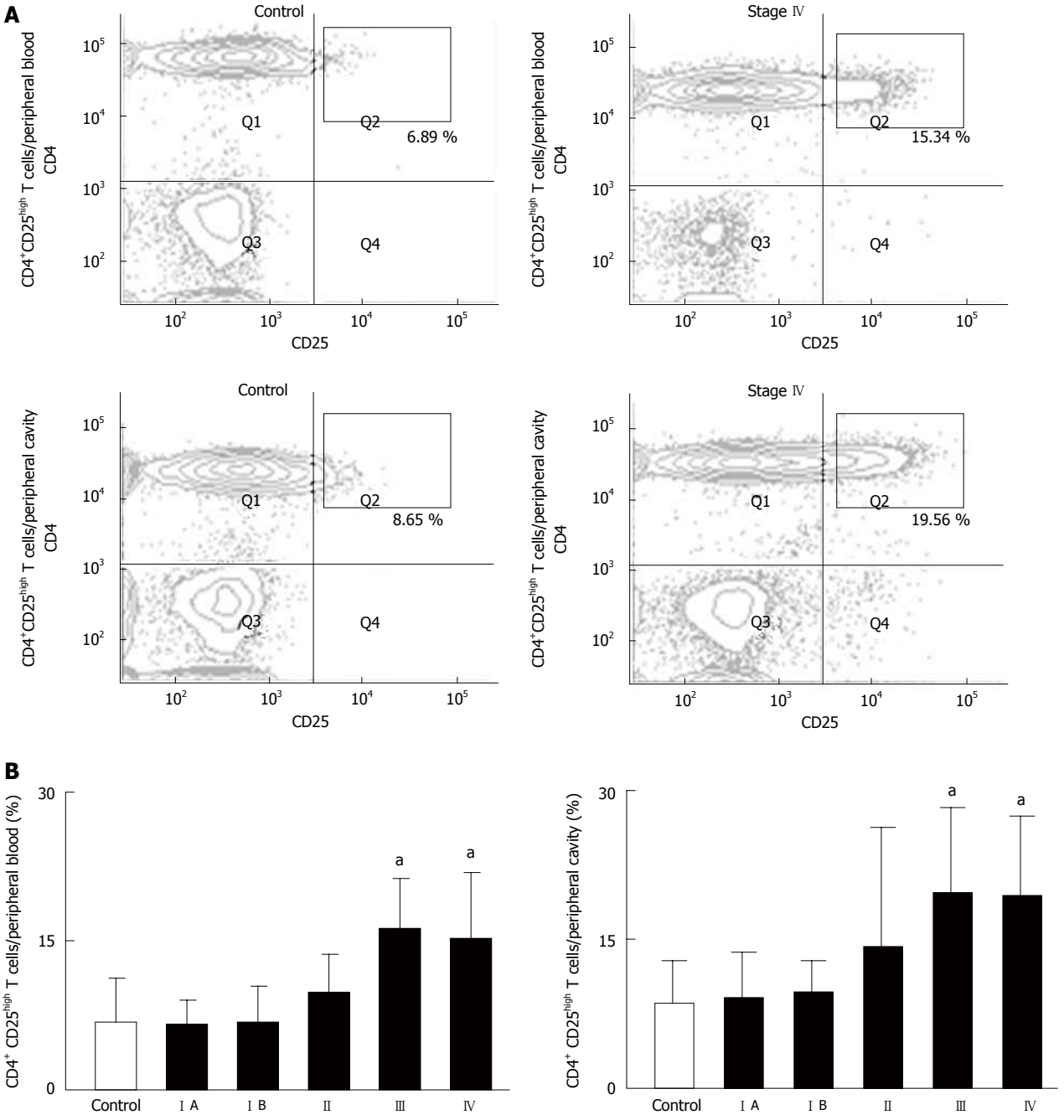

Figure 2 Analysis of lymphocyte populations in peripheral blood and the peritoneal cavity. A: The gating and counting of $CD4^+$ $CD25^{high}$ T cell population by flow cytometry; B: The percentage of $CD4^+$ $CD25^{high}$ T cells in the $CD4^+$ T cell population in peripheral blood and peritoneal lavage of patients at each stage of gastric cancer and control patients. Data are presented as the mean ± SD.

patients at advanced stage and control patients. Cancer progression may have an effect on the balance of the T cell population in the peritoneal cavity.

Immunological memory is demonstrated by following T cell subsets: lymph-node-homing cells lacking inflammatory and cytotoxic function (defined as central memory T cells, $CCR7^+$ $CD45RA^-$) and tissue-homing cells endowed with various effector functions (defined as effector memory T cells, $CCR7^-$ $CD45RA^-$). These two subsets allow for the division of labor among memory cells. Effector memory T cells represent a readily available pool of antigen-primed cells that can enter peripheral tissues to mediate inflammatory reactions or cytotoxicity, thus rapidly containing invasive pathogens and cancer antigens[11,28-31]. Our data show that $CD8^+$ effector memory T cells were rich in the peritoneal cavity. This indicates the migration of effector memory cells from the peripheral blood to local sites. However, in advanced cases, the frequency of $CD8^+$ effector memory cells in the peritoneal lavage was low. These results suggest that the peritoneal cavity exerts the local immune response, more than peripheral blood.

Natural killer T cells, a unique lymphocyte subpopulation, are characterized by the expression of invariant an-

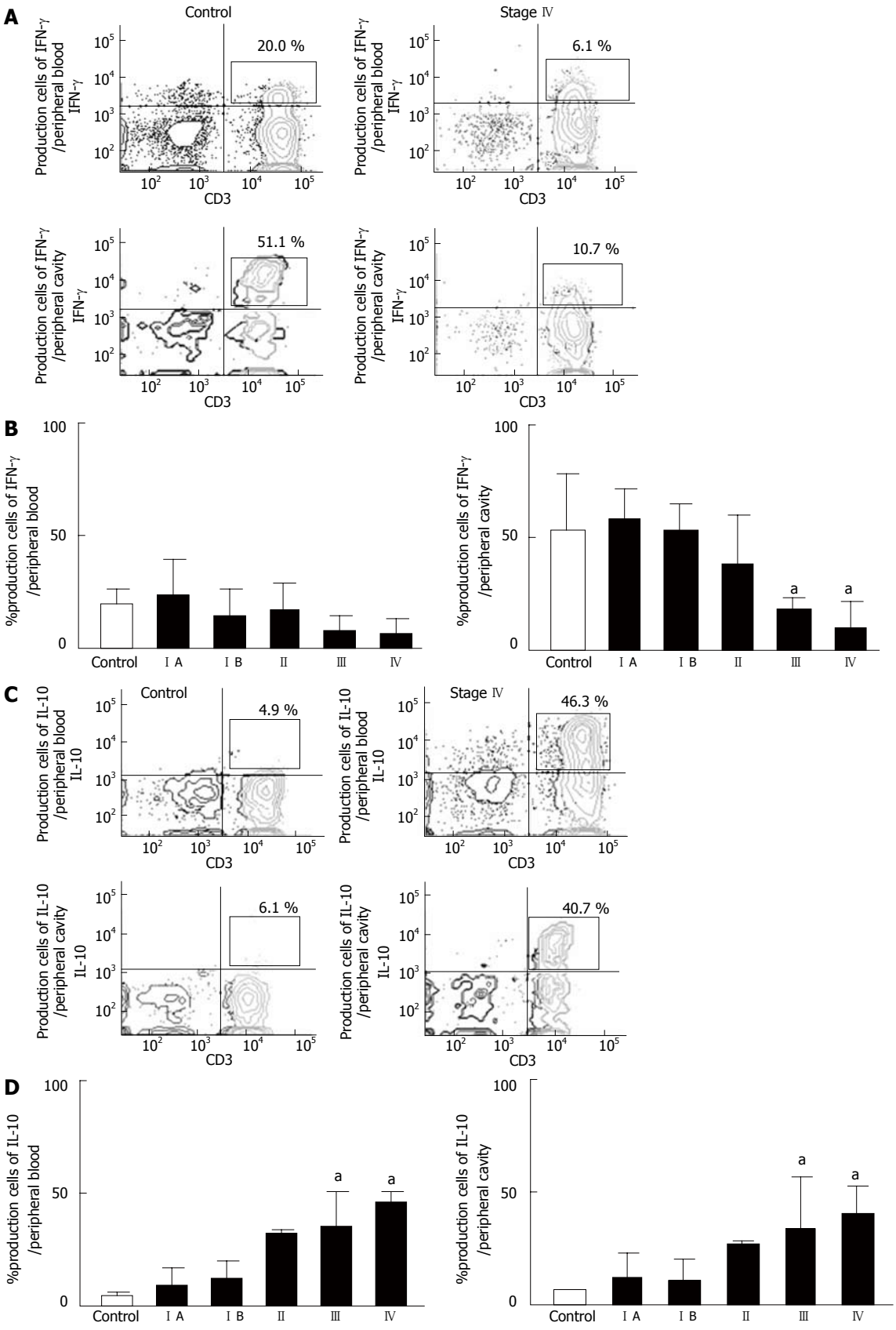

Figure 3 Cytokine production by lymphocytes. A: The gating and counting of the IFN-γ producing cell population by flow cytometry; B: The percentage of IFN-γ producing cells in the CD3$^+$ cell population stimulated with PMA + ionomycin in peripheral blood and peritoneal lavage of patients at each stage of gastric cancer and control patients Data are presented as the mean ± SD. The statistical analysis was performed by the Kruskal-Wallis test. After gating of CD3+ T cells, 10 000 events were analyzed. The production of IFN-γ in the peritoneal cavity was higher than that in the peripheral blood. The ratio of IFN-γ production cells in the peritoneal lavage was significantly lower in patients with advanced-stage than in controls [control *vs* stage Ⅳ: 51.1 (35.1-67.1) *vs* 10.7 (2.6-22.1), $^aP < 0.05$]; C: The gating and counting of the IL-10 producing cell population by flow cytometry; D: The percentage of IL-10 producing cells in the CD3$^+$ cells stimulated with PMA + ionomycin in peripheral blood and peritoneal lavage of patients at each stage of gastric cancer and control patients. Data are presented as the mean ± SD. The ratio of IL-10 producing cells in peripheral blood and intra-peritoneal lymphocytes was significantly higher in patients at advanced stage than in controls [control *vs* stage Ⅳ: 6.1 (3.94-8.25) *vs* 40.7 (18.35-63.0), $^aP < 0.05$].

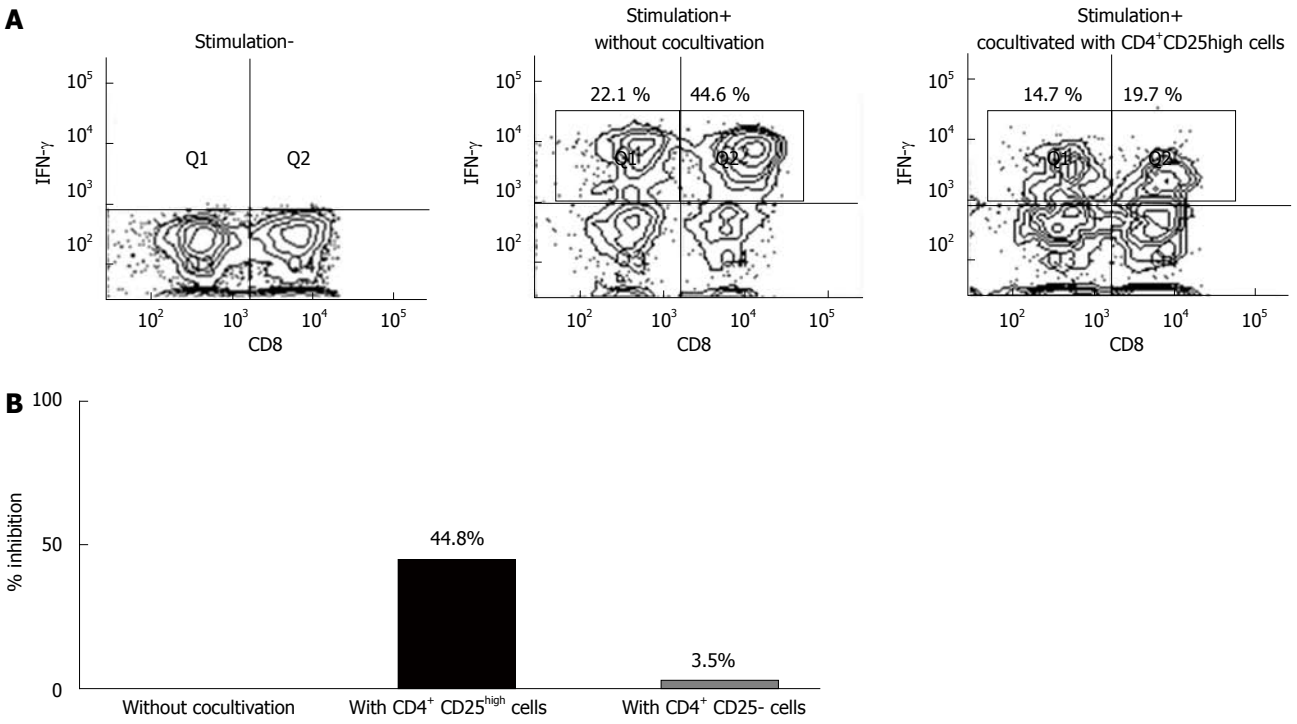

Figure 4 Cytokine assays of intra-peritoneal lymphocytes after co-cultivation with self-CD4$^+$CD25^{high}T cells. A: IFN-γ production in intra-peritoneal lymphocytes co-cultivated with self- CD4$^+$ CD25^{high} T cells; B: Either CD4$^+$ CD25^{high} T cells or CD4$^+$ CD25$^-$ T cells.

tigen receptors[12,13]. Natural killer T cells have been suggested to serve as a bridge between innate and acquired immunity[14,15]. However, the mechanisms underlying the anti-tumor effect of human natural killer T cell-mediated immunotherapy remain unclear so far. The frequency of natural killer T cells was lower in patients with stages Ⅲ and Ⅳ than in control patients. Therefore, a decrease in the number of natural killer T cells in the peritoneal cavity may be one aspect of the interaction between host-immunity and cancer progression.

Recent studies have shown that CD4$^+$ CD25^{high} foxp3$^+$ T cells exhibiting regulatory/suppressive properties are naturally present in humans[16-18]. The roles of regulatory T cells have been active topics of research in both basic and clinical immunology. Naturally-occurring regulatory T cells represent a small fraction (5%-6%) of the overall CD4$^+$ T cell population, and play an important role in down-regulation of the response of T cells to foreign and self antigens[31]. The depletion of this subset of regulatory T cells in normal hosts results in various autoimmune diseases because the host immune system is unchecked and attacks the body's own tissues[28]. Despite the importance of these cells in preventing autoimmune disease, their presence in the tumor microenvironment diminishes anti-tumor immune responses[32-36].

Within the CD4$^+$ T cell subset, there is a population of naturally occurring foxp3$^+$ T cells that are defined as regulatory T cells. These cells can be identified as CD4$^+$foxp3$^+$ T cells by flow cytometry. However, because foxp3 is intracellular and requires permeabilization of cells for detection by flow cytometry, regulatory T cells are isolated as CD4$^+$CD25^{high} T cells, which were shown to have functional suppressive abilities in our co-culture experiments[37]. In the present study, the mean percentage of CD4$^+$ CD25^{high} T cells in the peritoneal cavity in advanced gastric cancer patients was higher than that of control patients. After the co-cultivation of the self-CD4$^+$ CD25^{high} T cell population of intra-peritoneal lymphocytes, the production of IFN-γ was inhibited.

IFN-γ, a Th1 cytokine, not only exerts an anti-tumor effect, but also inhibits the proliferation of Th2 clones[19-20]. IL-10, a Th2 cytokine, suppresses the synthesis of Th1 cytokines such as IFN-γ[21-22]. This study showed that the production of intracellular cytokines in the peritoneal cavity was higher than that in the peripheral blood after appropriate stimulation. IFN-γ production was down-regulated in advanced cases, but not in the controls and stage Ⅰ patients. On the other hand, IL-10 production was up-regulated, which revealed the switch of Th1 and Th2 responses in the peritoneal cavity of these patients. IFN-γ production in intra-peritoneal lymphocytes was suppressed after co-cultivation with self-CD4$^+$ CD25^{high} T cells, but not CD4$^+$ CD25$^-$ T cells. Interestingly, the replacement of CD4$^+$ CD25$^-$ T cells for CD4$^+$ CD25^{high} T cells could recover the production of IFN-γ in intra-peritoneal lymphocytes.

COMMENTS

Background

The peritoneal cavity is a compartment in which immunological host-tumor interactions can occur. Neoplastic cell factors and biophylactic side factors such as immune reactions are interacting in the survival and development of micro-metastasis. However, the role of lymphocytes in the peritoneal cavity of gastric cancer patients is unclear.

Research frontiers

Clinical and experimental studies have established that leukocyte infiltrations around tumors promote the development or regression of solid tumors, but-whether the organ-specific cellular and molecular programs promote tumor growth or exhibit anti-tumor immunity by leukocytes are incompletely understood. Recent studies have shown that $CD4^+$ $CD25^{high}$ $foxp3^+$ T cells exhibiting regulatory/suppressive properties are naturally present in humans. The roles of regulatory T cells have been active topics of research in both basic and clinical immunology.

Innovations and breakthroughs

In most previous studies, malignant ascites have been a common source of immunological analysis. However, there are no reports describing the lymphocyte and cytokine production ability in peritoneal lavage from patients with gastric cancer at the time of gastrectomy. In the present study, $CD4^+$ $CD25^{high}$ T cells were found to be increased in the peritoneal cavity of advanced gastric cancer patients, but in the co-cultivation of the self- $CD4^+$ $CD25^{high}$ T cell population of intra-peritoneal lymphocytes, the production of IFN-γwas inhibited.

Applications

Peritoneal lavage samples from patients with gastric cancer are more susceptible than peripheral blood for monitoring the interaction between the host's immune system and tumor cells.

Terminology

Regulatory T cells: Regulatory T cells contribute to the maintenance of immunologic self-tolerance. Recent reports underscore that regulatory T cells not only play a role in the maintenance of immunotolerance but are also potent inhibitors of antitumor immune responses.

Peer review

The authors have investigated T-cells isolated from peripheral blood and peritoneal lavage in patients with gastric cancer and controls. Main findings are that in stage Ⅲ and Ⅳ gastric cancers the lavage fluid contains less CD8 memory cells, NKT cells and more $CD25^{high}$ regulatory T cells.

REFERENCES

1 **Huber V**, Fais S, Iero M, Lugini L, Canese P, Squarcina P, Zaccheddu A, Colone M, Arancia G, Gentile M, Seregni E, Valenti R, Ballabio G, Belli F, Leo E, Parmiani G, Rivoltini L. Human colorectal cancer cells induce T-cell death through release of proapoptotic microvesicles: role in immune escape. *Gastroenterology* 2005; **128**: 1796-1804

2 **Galon J**, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, Tosolini M, Camus M, Berger A, Wind P, Zinzindohoué F, Bruneval P, Cugnenc PH, Trajanoski Z, Fridman WH, Pagès F. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science* 2006; **313**: 1960-1964

3 **Susumu S**, Nagata Y, Ito S, Matsuo M, Valmori D, Yui K, Udono H, Kanematsu T. Cross-presentation of NY-ESO-1 cytotoxic T lymphocyte epitope fused to human heat shock cognate protein 70 by dendritic cells. *Cancer Sci* 2008; **99**: 107-112

4 **Koizumi K**, Hojo S, Akashi T, Yasumoto K, Saiki I. Chemokine receptors in cancer metastasis and cancer cell-derived chemokines in host immune response. *Cancer Sci* 2007; **98**: 1652-1658

5 **Tsujimoto H**, Ono S, Ichikura T, Matsumoto Y, Yamamoto J, Hase K. Roles of inflammatory cytokines in the progression of gastric cancer: friends or foes? *Gastric Cancer* 2010; **13**: 212-221

6 **Nan KJ**, Wei YC, Zhou FL, Li CL, Sui CG, Hui LY, Gao CG. Effects of depression on parameters of cell-mediated immunity in patients with digestive tract cancers. *World J Gastroenterol* 2004; **10**: 268-272

7 **Atanackovic D**, Block A, de Weerth A, Faltz C, Hossfeld DK, Hegewisch-Becker S. Characterization of effusion-infiltrating T cells: benign versus malignant effusions. *Clin Cancer Res* 2004; **10**: 2600-2608

8 **Cheever MA**, Greenberg PD, Fefer A. Specificity of adoptive chemoimmunotherapy of established syngeneic tumors. *J Immunol* 1980; **125**: 711-714

9 **Dudley ME**, Wunderlich JR, Robbins PF, Yang JC, Hwu P, Schwartzentruber DJ, Topalian SL, Sherry R, Restifo NP, Hubicki AM, Robinson MR, Raffeld M, Duray P, Seipp CA, Rogers-Freezer L, Morton KE, Mavroukakis SA, White DE, Rosenberg SA. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. *Science* 2002; **298**: 850-854

10 **Dudley ME**, Wunderlich JR, Yang JC, Sherry RM, Topalian SL, Restifo NP, Royal RE, Kammula U, White DE, Mavroukakis SA, Rogers LJ, Gracia GJ, Jones SA, Mangiameli DP, Pelletier MM, Gea-Banacloche J, Robinson MR, Berman DM, Filie AC, Abati A, Rosenberg SA. Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. *J Clin Oncol* 2005; **23**: 2346-2357

11 **Sallusto F**, Lenig D, Förster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* 1999; **401**: 708-712

12 **Taniguchi M**, Harada M, Kojo S, Nakayama T, Wakao H. The regulatory role of Valpha14 NKT cells in innate and acquired immune response. *Annu Rev Immunol* 2003; **21**: 483-513

13 **Brigl M**, Brenner MB. CD1: antigen presentation and T cell function. *Annu Rev Immunol* 2004; **22**: 817-890

14 **Taniguchi M**, Seino K, Nakayama T. The NKT cell system: bridging innate and acquired immunity. *Nat Immunol* 2003; **4**: 1164-1165

15 **Motohashi S**, Nakayama T. Clinical applications of natural killer T cell-based immunotherapy for cancer. *Cancer Sci* 2008; **99**: 638-645

16 **Sakaguchi S**. Naturally arising CD4+ regulatory t cells for immunologic self-tolerance and negative control of immune responses. *Annu Rev Immunol* 2004; **22**: 531-562

17 **Linehan DC**, Goedegebuure PS. CD25+ CD4+ regulatory T-cells in cancer. *Immunol Res* 2005; **32**: 155-168

18 **Imai H**, Saio M, Nonaka K, Suwa T, Umemura N, Ouyang GF, Nakagawa J, Tomita H, Osada S, Sugiyama Y, Adachi Y, Takami T. Depletion of CD4+CD25+ regulatory T cells enhances interleukin-2-induced antitumor immunity in a mouse model of colon adenocarcinoma. *Cancer Sci* 2007; **98**: 416-423

19 **Fernandez-Botran R**, Sanders VM, Mosmann TR, Vitetta ES. Lymphokine-mediated regulation of the proliferative response of clones of T helper 1 and T helper 2 cells. *J Exp Med* 1988; **168**: 543-558

20 **Rayman P**, Wesa AK, Richmond AL, Das T, Biswas K, Raval G, Storkus WJ, Tannenbaum C, Novick A, Bukowski R, Finke J. Effect of renal cell carcinomas on the development of type 1 T-cell responses. *Clin Cancer Res* 2004; **10**: 6360S-6366S

21 **Fiorentino DF**, Zlotnik A, Vieira P, Mosmann TR, Howard M, Moore KW, O'Garra A. IL-10 acts on the antigen-presenting cell to inhibit cytokine production by Th1 cells. *J Immunol* 1991; **146**: 3444-3451

22 **Bai XF**, Zhu J, Zhang GX, Kaponides G, Höjeberg B, van der Meide PH, Link H. IL-10 suppresses experimental autoimmune neuritis and down-regulates TH1-type immune responses. *Clin Immunol Immunopathol* 1997; **83**: 117-126

23 **Japanese Gastric Cancer Association.** Japanese classification of gastric carcinoma--2nd English edition--response assessment of chemotherapy and radiotherapy for gastric carcinoma: clinical criteria. *Gastric Cancer* 2001; **4**: 1-8

24 **Olszewski WL**, Kubicka U, Tarnowski W, Bielecki K, Ziolkowska A, Wesolowska A. Activation of human peritoneal immune cells in early stages of gastric and colon cancer. *Surgery* 2007; **141**: 212-221

25 **Mori T**, Shimizu M, Iwaguchi T. Immunological characterization and clinical significance of low mobility cells appearing in the peripheral blood mononuclear cells of cancer patients. *Eur J Cancer Clin Oncol* 1988; **24**: 1463-1469

26 **Marutsuka T**, Shimada S, Shiomori K, Hayashi N, Yagi Y, Yamane T, Ogawa M. Mechanisms of peritoneal metastasis after operation for non-serosa-invasive gastric carcinoma: an ultrarapid detection system for intraperitoneal free cancer cells and a prophylactic strategy for peritoneal metastasis. *Clin Cancer Res* 2003; **9**: 678-685

27 **Jung M**, Jeung HC, Lee SS, Park JY, Hong S, Lee SH, Noh SH, Chung HC, Rha SY. The clinical significance of ascitic fluid CEA in advanced gastric cancer with ascites. *J Cancer Res Clin Oncol* 2010; **136**: 517-526

28 **Sallusto F**, Geginat J, Lanzavecchia A. Central memory and effector memory T cell subsets: function, generation, and maintenance. *Annu Rev Immunol* 2004; **22**: 745-763

29 **Berger C**, Jensen MC, Lansdorp PM, Gough M, Elliott C, Riddell SR. Adoptive transfer of effector CD8+ T cells derived from central memory cells establishes persistent T cell memory in primates. *J Clin Invest* 2008; **118**: 294-305

30 **Ye SW**, Wang Y, Valmori D, Ayyoub M, Han Y, Xu XL, Zhao AL, Qu L, Gnjatic S, Ritter G, Old LJ, Gu J. Ex-vivo analysis of CD8+ T cells infiltrating colorectal tumors identifies a major effector-memory subset with low perforin content. *J Clin Immunol* 2006; **26**: 447-456

31 **Wang HY**, Wang RF. Regulatory T cells and cancer. *Curr Opin Immunol* 2007; **19**: 217-223

32 **Nishikawa H**, Kato T, Hirayama M, Orito Y, Sato E, Harada N, Gnjatic S, Old LJ, Shiku H. Regulatory T cell-resistant CD8+ T cells induced by glucocorticoid-induced tumor necrosis factor receptor signaling. *Cancer Res* 2008; **68**: 5948-5954

33 **Curiel TJ**, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, Evdemon-Hogan M, Conejo-Garcia JR, Zhang L, Burow M, Zhu Y, Wei S, Kryczek I, Daniel B, Gordon A, Myers L, Lackner A, Disis ML, Knutson KL, Chen L, Zou W. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat Med* 2004; **10**: 942-949

34 **Wing K**, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, Fehervari Z, Nomura T, Sakaguchi S. CTLA-4 control over Foxp3+ regulatory T cell function. *Science* 2008; **322**: 271-275

35 **Onishi Y**, Fehervari Z, Yamaguchi T, Sakaguchi S. Foxp3+ natural regulatory T cells preferentially form aggregates on dendritic cells in vitro and actively inhibit their maturation. *Proc Natl Acad Sci USA* 2008; **105**: 10113-10118

36 **Stanzer S**, Dandachi N, Balic M, Resel M, Samonigg H, Bauernhofer T. Resistance to apoptosis and expansion of regulatory T cells in relation to the detection of circulating tumor cells in patients with metastatic epithelial cancer. *J Clin Immunol* 2008; **28**: 107-114

37 **Gnjatic S**, Altorki NK, Tang DN, Tu SM, Kundra V, Ritter G, Old LJ, Logothetis CJ, Sharma P. NY-ESO-1 DNA vaccine induces T-cell responses that are suppressed by regulatory T cells. *Clin Cancer Res* 2009; **15**: 2130-2139

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ORIGINAL ARTICLE

Curcumin prevents indomethacin-induced gastropathy in rats

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Abstract

AIM: To investigate the effects of curcumin on gastric microcirculation and inflammation in rats with indomethacin-induced gastric damage.

METHODS: Male Sprague-Dawley rats were randomly divided into three groups. Group 1 (control group, *n* = 5) was fed with olive oil and 5% $NaHCO_3^-$ (vehicle). Group 2 [indomethacin (IMN) group, *n* = 5] was fed with olive oil 30 min prior to indomethacin 150 mg/kg body weight (BW) dissolved in 5% $NaHCO_3^-$ at time 0th and 4th h. Group 3 (IMN + Cur group, *n* = 4) was fed with curcumin 200 mg/kg BW dissolved in olive oil 0.5 mL, 30 min prior to indomethacin at 0th and 4th h. Leukocyte-endothelium interactions at postcapillary venules were recorded after acridine orange injection. Blood samples were determined for intercellular adhesion molecule (ICAM)-1 and tumor necrosis factor (TNF)-α levels using enzyme linked immunosorbent assay method. Finally, the stomach was removed for histopathological examination for gastric lesions and grading for neutrophil infiltration.

RESULTS: In group 2, the leukocyte adherence in postcapillary venules was significantly increased compared to the control group (6.40 ± 2.30 cells/frame *vs* 1.20 ± 0.83 cells/frame, *P* = 0.001). Pretreatment with curcumin caused leukocyte adherence to postcapillary venule to decline (3.00 ± 0.81 cells/frame *vs* 6.40 ± 2.30 cells/frame, *P* = 0.027). The levels of ICAM-1 and TNF-α increased significantly in the indomethacin-treated group compared with the control group (1106.50 ± 504.22 pg/mL *vs* 336.93 ± 224.82 pg/mL, *P* = 0.011 and 230.92 ± 114.47 pg/mL *vs* 47.13 ± 65.59 pg/mL, *P* = 0.009 respectively). Pretreatment with curcumin significantly decreased the elevation of ICAM-1 and TNF-α levels compared to treatment with indomethacin alone (413.66 ± 147.74 pg/mL *vs* 1106.50 ± 504.22 pg/mL, *P* = 0.019 and 58.27 ± 67.74 pg/mL *vs* 230.92 ± 114.47 pg/mL, *P* = 0.013 respectively). The histological appearance of the stomach in the control group was normal. In the indomethacin-treated group, the stomachs showed a mild to moderate neutrophil infiltration score. Gastric lesions were erosive and ulcerative. In rats treated with indomethacin and curcumin, stomach histopathology improved and showed only a mild neutrophil infiltration score and fewer erosive lesions in the gastric mucosa.

CONCLUSION: The results indicate that curcumin prevents indomethacin-induced gastropathy through the improvement of gastric microcirculation by attenuating the level of ICAM-1 and TNF-α.

Key words: Curcumin; Nonsteroidal anti-inflammatory drugs; Gastric damage; Gastric microcirculation; Intercellular adhesion molecule-1; Tumor necrosis factor-α

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INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most commonly prescribed medications worldwide. However, NSAIDs have adverse effects on the gastric mucosa, resulting in various clinical presentations, ranging from nonspecific dyspepsia to ulceration, upper gastrointestinal bleeding, and death, summarized by the term "NSAID gastropathy"[1]. NSAIDs-induced gastric damage is the major side effect of this kind of drug[2].

The main action of NSAIDs is to inhibit prostaglandin synthesis. There is substantial evidence supporting the view that the ulcerogenic effect of this medication correlates with its ability to suppress prostaglandin synthesis[3-5]. Endogenous prostaglandins normally regulate mucosal blood flow, epithelial cell proliferation, epithelial restitution, mucosal immunocyte function, mucus and bicarbonate secretion, and basal acid secretion[6]. Therefore, decreases in prostaglandins, protective factors for ulcer formation, lead to gastric mucosal injury.

Animal studies have shown that neutrophil adherence to the endothelium of the gastric microcirculation is critical in NSAIDs injury[7]. Neutrophil adherence damages the mucosa by producing oxygen-free radicals, releasing proteases, and obstructing capillary blood flow. NSAIDs might induce the synthesis of tumor necrosis factor (TNF)-α and leukotrienes[8,9]. These inflammatory mediators subsequently stimulate neutrophil adherence by the upregulation of adhesion molecules[10].

NSAID administration in rats caused a rapid and significant increase in adhesion between neutrophils and vascular endothelial cells in both gastric and mesenteric venules[11-13]. This was dependent on intercellular adhesion molecule (ICAM)-1 expression on the endothelium and CD11/CD18 expression on the leukocyte[14,15]. Interestingly, Andrews *et al*[10] recently reported that administration of aspirin or indomethacin to rats resulted in a significant increase in ICAM-1 expression in the gastric microcirculation.

Curcuma, a genus in the plant family of Zingiberacea, is the biological source for curcuminoids, including curcumin. Curcuma longa, the yellow tuberous root referred to as turmeric, was taken from India to Southeast Asia[16]. The yellow pigmented fraction of Curcuma longa contains curcuminoids, which are chemically related to its principal ingredient, curcumin[16]. It possesses a broad range of pharmacological activities, including antioxidant, anti-carcinogenic, wound-healing, and anti-inflammatory effects[17-19]. There are currently limited studies investigating the effect of curcumin on NSAIDs-induced gastric damage. The aim of this study was to investigate the anti-inflammation effect of curcumin on indomethacin-induced gastric damage in rats.

MATERIALS AND METHODS

Animal preparation and curcumin preparation

Male Sprague-Dawley rats weighing 180-220 g, purchased from the National Laboratory Animal Center, Mahidol University, Salaya (n = 18), Nakorn pathom, were used in this study. All rats were kept in a controlled temperature room at 25 ± 1 ℃ under standard conditions (12 h day-night rhythm). They were cared for in accordance with the Ethical Committee, Faculty of Medicine, Chulalongkorn University, Thailand. Curcumin powder (Cayman Chemical Company, United States) was suspended in olive oil.

Experimental protocol

All rats were fasted, with free access to water ad libitum, for 22-24 h before the experiment. They were randomly divided into three experimental groups. Group 1 (control, n = 6): Rats were fed with olive oil 30 min prior to 5% sodium bicarbonate 1 mL orally *via* an intragastric tube at time 0th and 4th h. Group 2 [indomethacin (IMN), n = 6]: Rats were fed with olive oil 30 min prior to indomethacin (150 mg/kg body weight in 5% sodium bicarbonate 1 mL orally *via* an intragastric tube) at time 0th and 4th h. Group 3 (IMN + Cur, n = 6): Rats were fed with curcumin (200 mg/kg body weight dissolved in olive oil 0.5 mL) 30 min prior to indomethacin [150 mg/kg body weight (BW) dissolved in 5% sodium bicarbonate 1 mL orally *via* an intragastric tube] at time 0th and 4th h.

After 8 h 30 min, animals were anesthetized with intraperitoneal injection of thiopental (50 mg/kg body weight). After tracheostomy, the carotid artery and jugular vein were cannulated for blood pressure measurement using a polygraph and for the administration of a fluorescent marker; acridine orange was infused intravenously (Sigma chemical Co., United States, 0.5 mg/kg BW/min). The abdominal wall was incised and the stomach was extended and fixed. Leukocyte adherence in the stomach was observed by intravital fluorescence microscopy. At the end of the experiment, blood samples were collected for ICAM-1 and TNF-α determination using enzyme linked immunosorbent assay (ELISA) methods. The stomach was cut and fixed in 10% formalin solution to inspect the histopathology.

Study of the interaction between leukocytes and endothelial cells in postcapillary venule

It has been stated that NSAIDs-induced leukocyte adherence could contribute to the pathogenesis of gastric

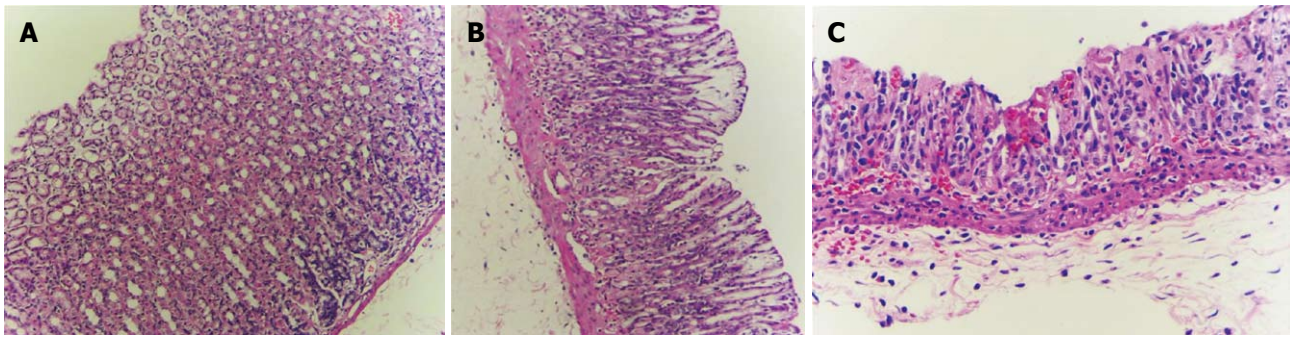

Figure 1 Hematoxylin-eosin stained stomach sections (× 200). A: The control group showed normal stomach histopathology; B: The indomethacin treated group showed gastric ulcer formation and infiltration of inflammatory cells; C: The curcumin treated group showed a reduced degree of gastric ulcer formation and inflammation.

mucosal injury. To visualize leukocytes, acridine orange was infused intravenously (0.3 mg/kg body weight). The number of leukocyte adhesions was recorded using a video recorder. Videotape of each experiment was replayed and leukocyte adherence was monitored. Most leukocytes were adhered to the postcapillary venule (about 15-30 μm in diameter). Leukocytes were considered adherent to the vessel endothelium if they remained stationary for 30 s or longer. Adherent leukocytes were expressed as the number of leukocyte adherences per frame of view, as previously described[20].

Determination of serum cytokine levels

After the experiment, blood samples were taken by cardiac puncture, and allowed to clot overnight before centrifuging at approximately 2000 × *g*. Serum was stored at -80 ℃ for determining ICAM-1 and TNF-α levels by ELISA kit (R and D systems).

Histopathological examination

Samples of the stomach were excised and transferred to formalin. The samples were subsequently processed by routine techniques before embedding in paraffin. Sections were cut at the thickness of 5 μm and stained with hematoxylin and eosin (HE), as previously described[20]. One pathologist performed all the histopathological examinations. All histopathological changes were observed under a light microscope. The neutrophil infiltration score in each section was graded according previously determined criteria[21].

Statistical analysis

All data were presented as mean ± SD. To compare data among all groups of animals, one-way analysis of variance (one-way ANOVA) and Duncan comparisons were employed. All statistical tests were performed using SPSS for Windows version 13.0 (SPSS Inc., Chicago, IL, United States). Differences were considered statistically significant at $P < 0.05$.

RESULTS

Histopathological examination

The histological appearance of the stomach in the control group (Figure 1) was normal. In the indomethacin treated group, the stomachs showed mild to moderate gastric mucosal injury. Gastric lesions were erosive and ulcerative. In rats treated with indomethacin and curcumin, the stomach histopathology improved and showed only mild gastric mucosal injury and reduced amounts of erosive lesions in the gastric mucosa. The summary of infiltration of inflammatory cells and gastric lesions are shown in Table 1.

Table 1 Summary of the infiltration of inflammatory cells and erosion in all groups ($n = 6$)

Experimental group	Neutrophil infiltration[1]				Pathology		
	0	1	2	3	No erosion	Erosion	Ulcer
Control	6	-	-	-	6	-	-
IMN	-	3	3	-	-	2	4
IMN + Cur	4	2	-	-	4	2	-

[1]The severity of neutrophil infiltration was graded as: 0: No neutrophil infiltration; 1: Neutrophil infiltration found in 1/3 of gastric mucosal layer; 2: Neutrophil infiltration found in 2/3 of gastric mucosal layer; 3: Neutrophil infiltration found in the muscularis mucosae of gasm. IMN: Indomethacin.

Interaction between leukocytes and endothelial cells

After gastric injury was induced by the administration of indomethacin, leukocyte adherence to endothelial cells of postcapillary venules (15-30 μm in diameter) was observed under intravital fluorescence microscopy, 10-15 min after acridine orange injection (Figure 2). The number of leukocytes adhering to postcapillary venules for 30 s or longer was counted. The mean number of leukocyte adherences in the IMN group without curcumin treatment was significantly higher than in the control group (6.40 ± 2.30 cells/frame *vs* 1.20 ± 0.83 cells/frame, $P = 0.001$).

The number of leukocyte adherences significantly decreased after pretreatment with curcumin as compared to the IMN group (3.00 ± 0.81 cells/frame *vs* 6.40 ± 2.30 cells/frame, $P = 0.027$).

Changes in ICAM-1 levels

The levels of ICAM-1 increased significantly in the indomethacin treated group compared with the control group

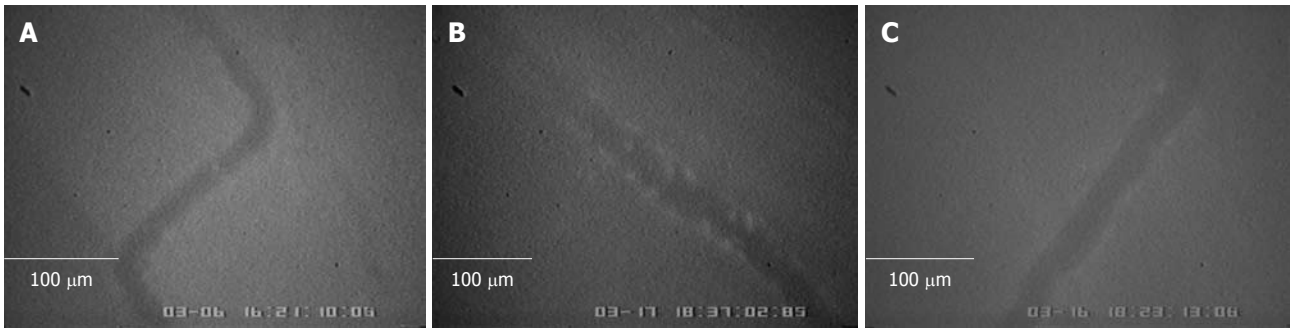

Figure 2 Intravital microscopic (× 40) images of leukocyte adherence on vascular endothelium of postcapillary venules 10-15 min after acridine orange injection in the control group (A), IMN group (B), and IMN + Cur group (C). IMN: Indomethacin.

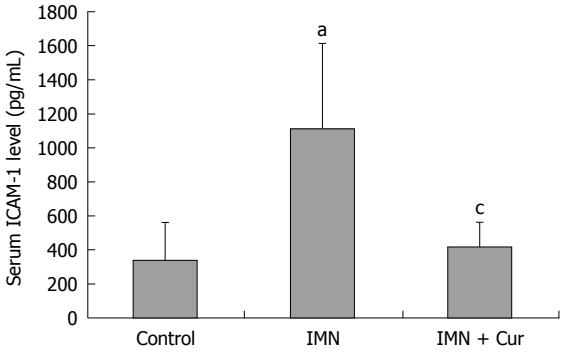

Figure 3 Serum intercellular adhesion molecule-1 levels in all groups. All data are expressed as mean ± SD. The mean intercellular adhesion molecule (ICAM)-1 levels were significantly higher in the indomethacin treated group (IMN) when compared with the control group ($^aP < 0.05$). Pretreatment with curcumin significantly decreased ICAM-1 level when compared with indomethacin ($^cP < 0.05$). IMN: Indomethacin.

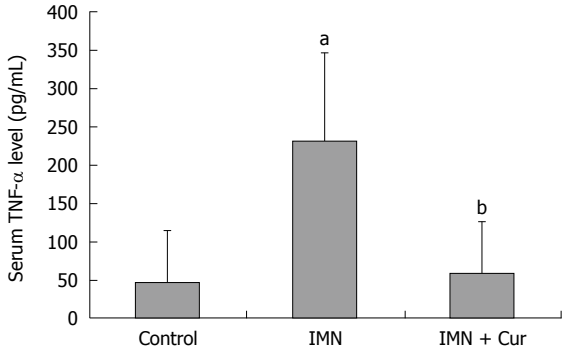

Figure 4 Serum tumor necrosis factor-alpha level in all groups. All data are expressed as mean ± SD. The mean of tumor necrosis factor (TNF)-α levels increased in the indomethacin treated group when compared with the control group ($^aP = 0.009$). Pretreatment with curcumin decreased serum of TNF-α when compared with indomethacin alone ($^bP = 0.013$). IMN: Indomethacin.

(1106.50 ± 504.22 pg/mL *vs* 336.93 ± 224.82 pg/mL, $P = 0.011$). Pretreatment with curcumin markedly decreased the elevation of the ICAM-1 level compared with the indomethacin treated group (413.66 ± 147.74 pg/mL *vs* 1106.50 ± 504.22 pg/mL, $P = 0.019$) (Figure 3).

Changes in TNF-α levels

The level of TNF-α markedly increased in the indomethacin treated group compared with the control group (230.92 ± 114.47 pg/mL *vs* 47.13 ± 65.59 pg/mL, $P = 0.009$). Pretreatment with curcumin decreased the elevation of TNF-α levels compared with the indomethacin treated group (58.27 ± 67.74 pg/mL *vs* 230.92 ± 114.47 pg/mL, $P = 0.013$) (Figure 4).

DISCUSSION

In the present study, we investigated the effects of curcumin on indomethacin-induced gastric damage in rats. The results clearly demonstrated that curcumin administration prevented the ulcerogenic effect of indomethacin, possibly through its anti-inflammatory action. Evidence suggests that NSAIDs-induced gastric ulceration is a neutrophil-dependent process. NSAIDs administration to rats caused a rapid and significant increase in adhesion between neutrophils and vascular endothelial cells in both the gastric and mesenteric venules[11,12]. Indeed, monoclonal antibodies that blocked NSAID-induced neutrophil adherence to vascular endothelium could significantly alleviate NSAID- induced gastric mucosal injury[7,15,22]. Neutrophils play an important role in the development of inflammation and tissue injury by releasing a variety of inflammatory mediators[23,24]. These inflammatory mediators are capable of producing tissue injury; therefore, they may be involved in the pathogenesis of indomethacin-induced gastric mucosal injury[25]. Furthermore, adhesion molecules expressed on activated neutrophils, such as CD11b and CD18, have been shown to play an important role in neutrophil-induced tissue injury[14].

Moreover, NSAIDS are believed to have the effect on nuclear translocation of nuclear factor (NF)-κB, which modulates the expression of several adhesions molecules, including ICAM-1[26]. ICAM-1, one of the major adhesion molecules, plays a pivotal role in the inflammatory reaction by increasing leukocyte adhesion to endothelium and promoting transendothelial migration of leukocytes to inflammatory sites[27]. Another important mechanism that induces ICAM-1 expression is the increment of TNF-α levels[27,28]. The inhibitory effect of NSAIDs on COX-2 leads reduced prostaglandin E2 (PGE2) levels. Thus, TNF-α production, which is normally inhibited

by PGE2, increases[28]. TNF-α is an important mediator causing NSAIDs induced gastropathy. Apart from its effect on adhesion molecules, TNF-α may have the ability to activate pro-apoptosis caspases, which regulate gastric epithelial cells apoptosis in NSAID-treated rats[27].

A previous study demonstrated that indomethacin administration caused significantly elevated TNF-α levels in rats[8]. Moreover, pretreatment with anti- TNF-α, dexamethasone, and PGE2 could prevent the increases in gastric mucosal injuries and TNF-α levels. Similarly, we found significant rises in TNF-α and ICAM-1 levels in the serum of the indomethacin-treated group compared to the control. Other formulations of NSAIDs could also increase TNF- α production. A study by Jainu *et al*[29] noted a significant increase in TNF-α, IL-1β and NOS-2 activity in aspirin-administered rats. The elevations of inflammatory mediators and adhesion molecules in serum correlated with the pathological findings of gastric mucosa and the numbers of adhered leukocytes in the gastric microcirculation. These were also true for curcumin-treated group. Pretreatment with curcumin could significantly reduce TNF-α and ICAM-1 levels in the serum, accompanied by an improvement in gastric mucosal inflammation and leukocyte adhesion.

Curcumin, a substance rich in phenolics, is known to possess antioxidant properties. Curcumin reduces gastric injury induced by NSAIDs[30]. It has been reported that curcumin can decrease gastric injury by preventing the peroxidase inactivation effect of indomethacin and scavenging reactive oxygen produced by this enzyme[30]. Several studies showed that curcumin is also an anti-inflammatory substance, with an inhibitory effect on transcription factor NF-κB activation. NF-κB is required for the expression of many genes linked with the host immune response, such as ICAM-1, TNF-α, IL-1β, and iNOS[31]. Cytoplasmic NF-κB is complexed with its inhibitor IkB and is, therefore, inactive. The cytokine-mediated activation of NF-κB requires activation of various kinases, which ultimately leads to the phosphorylation and degradation of IkB. Several beneficial effects of curcumin are consistent with its ability to inhibit the activity of NF-κB[32-34]. Singh *et al*[31] observed that curcumin inhibited NF-κB activation pathway after the convergence of various stimuli mediated by protein tyrosine kinase, protein kinase, and ubiquitin conjugation enzymes, but before the phosphorylation and subsequent release of IkB complexed to NF-κB. Jobin *et al*[35] and Plummer *et al*[36] examined the modulatory potential of curcumin on NF-κB signaling pathways and found that curcumin prevented phosphorylation of IkB by inhibiting the activation of IkB-kinase (IKKs). Our previous study demonstrated that *Helicobacter pylori*-induced gastric inflammation in rats is associated with increased NF-κB activation and macromolecular leakage, which can be reduced by curcumin[37]. In this study, 200 mg/kg curcumin was a sufficient dose for reducing gastric epithelial NF-κB p65 expression and mucosal macromolecular leakage. Despite its inconclusive mechanism of action, we clearly demonstrated that curcumin has a protective and beneficial effect on NSAIDs-induced gastropathy in rats. Further studies on the expression of inflammatory mediators and adhesion molecules in the gastric mucosa are necessary to demonstrate the exact curative effect of curcumin on NSAID-induced gastric pathology. Clinical studies might also be needed to verify the protective effect of curcumin in humans.

In conclusion, NSAIDs could induce gastric injury through increases in inflammatory cytokines and leukocyte adhesions. Curcumin, an anti-oxidant herbal substance, could prevent these adverse events and might be used as a preventive method for NSAIDs-induced gastropathy.

COMMENTS

Background

Nonsteroidal anti-inflammatory drugs (NSAIDs)-induced gastric damage is the major side effect of this kind of medication. Although the underlying pathogenesis of NSAIDs-induced gastric damage is unclear, neutrophils are believed to play an important role in the development of gastric inflammation and injury following NSAID administration. Curcumin possesses several biological activities, including an anti-inflammatory effect. Authors postulated that curcumin, acting through nuclear factor (NF)-κB inhibition, could reduce the production of adhesion molecules and inflammatory cytokines, resulting in the amelioration of gastric injury in NSAIDs-induced gastropathy in rats.

Research frontiers

Curcumin (diferuloylmethane) is an active ingredient of *Curcuma longa* (turmeric), which exerts many biological activities by the inhibition of NF-κB-mediated reactions. NSAIDs can cause gastric mucosal damage through an increase in leukocyte-endothelial adhesions and the release of inflammatory mediators, leading to the free radical production. This study demonstrated an improvement in gastric mucosal damage and decreases in leukocyte adhesions, and intercellular adhesion molecule 1 and tumor necrosis factor (TNF)-α production after curcumin administration in the indomethacin-treated group.

Innovations and breakthroughs

The previous study showed that curcumin is an anti-inflammatory agent and can inhibit NF-κB activation in an *in vitro* study. However, it is not known whether curcumin's anti-inflammatory effects will help prevent NSAIDs-induced gastropathy *in vivo*. In this study, authors investigated the protective effect of curcumin in indomethacin-induced gastric damage in rats. Authors found that curcumin could alleviate indomethacin-induced gastric injury *via* a decrease in leukocyte adhesions and TNF-α production.

Applications

Curcumin might be used as a new protective agent for NSAIDs-induced gastric damage in clinical use.

Terminology

NSAIDs gastropathy: NSAIDs are well-known for their adverse effects on gastric mucosa, resulting in various clinical presentations, ranging from nonspecific dyspepsia to ulceration, upper gastrointestinal bleeding and death, summarized by the term "NSAID gastropathy".

Peer review

This is an interesting study of the effects of curcumin on indomethacin-induced gastric damage in rats. The results clearly demonstrated that curcumin administration prevented the ulcerogenic effect of indomethacin, possibly through its anti-inflammatory action.

REFERENCES

1 **Becker JC**, Domschke W, Pohle T. Current approaches to prevent NSAID-induced gastropathy--COX selectivity and beyond. *Br J Clin Pharmacol* 2004; **58**: 587-600
2 **Wolfe MM**, Lichtenstein DR, Singh G. Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs. *N Engl J Med* 1999; **340**: 1888-1899
3 **Vane JR**. Inhibition of prostaglandin synthesis as a mecha-

nism of action for aspirin-like drugs. *Nat New Biol* 1971; **231**: 232-235
4 **Whittle BJ**. Temporal relationship between cyclooxygenase inhibition, as measured by prostacyclin biosynthesis, and the gastrointestinal damage induced by indomethacin in the rat. *Gastroenterology* 1981; **80**: 94-98
5 **Rainsford KD**, Willis C. Relationship of gastric mucosal damage induced in pigs by antiinflammatory drugs to their effects on prostaglandin production. *Dig Dis Sci* 1982; **27**: 624-635
6 **Wallace JL**. Nonsteroidal anti-inflammatory drugs and gastroenteropathy: the second hundred years. *Gastroenterology* 1997; **112**: 1000-1016
7 **Wallace JL**, Keenan CM, Granger DN. Gastric ulceration induced by nonsteroidal anti-inflammatory drugs is a neutrophil-dependent process. *Am J Physiol* 1990; **259**: G462-G467
8 **Appleyard CB**, McCafferty DM, Tigley AW, Swain MG, Wallace JL. Tumor necrosis factor mediation of NSAID-induced gastric damage: role of leukocyte adherence. *Am J Physiol* 1996; **270**: G42-G48
9 **Rainsford KD**. Microvascular injury during gastric mucosal damage by anti-inflammatory drugs in pigs and rats. *Agents Actions* 1983; **13**: 457-460
10 **Andrews FJ**, Malcontenti-Wilson C, O'Brien PE. Effect of nonsteroidal anti-inflammatory drugs on LFA-1 and ICAM-1 expression in gastric mucosa. *Am J Physiol* 1994; **266**: G657-G664
11 **Wallace JL**, McKnight W, Miyasaka M, Tamatani T, Paulson J, Anderson DC, Granger DN, Kubes P. Role of endothelial adhesion molecules in NSAID-induced gastric mucosal injury. *Am J Physiol* 1993; **265**: G993-G998
12 **Asako H**, Kubes P, Wallace J, Gaginella T, Wolf RE, Granger DN. Indomethacin-induced leukocyte adhesion in mesenteric venules: role of lipoxygenase products. *Am J Physiol* 1992; **262**: G903-G908
13 **Asako H**, Kubes P, Wallace J, Wolf RE, Granger DN. Modulation of leukocyte adhesion in rat mesenteric venules by aspirin and salicylate. *Gastroenterology* 1992; **103**: 146-152
14 **Wallace JL**, Arfors KE, McKnight GW. A monoclonal antibody against the CD18 leukocyte adhesion molecule prevents indomethacin-induced gastric damage in the rabbit. *Gastroenterology* 1991; **100**: 878-883
15 **Wallace JL**, McCafferty DM, Carter L, McKnight W, Argentieri D. Tissue-selective inhibition of prostaglandin synthesis in rat by tepoxalin: anti-inflammatory without gastropathy? *Gastroenterology* 1993; **105**: 1630-1636
16 **Joe B**, Vijaykumar M, Lokesh BR. Biological properties of curcumin-cellular and molecular mechanisms of action. *Crit Rev Food Sci Nutr* 2004; **44**: 97-111
17 **Toda S**, Miyase T, Arichi H, Tanizawa H, Takino Y. Natural antioxidants. III. Antioxidative components isolated from rhizome of Curcuma longa L. *Chem Pharm Bull* (Tokyo) 1985; **33**: 1725-1728
18 **Srimal RC**, Dhawan BN. Pharmacology of diferuloyl methane (curcumin), a non-steroidal anti-inflammatory agent. *J Pharm Pharmacol* 1973; **25**: 447-452
19 **Satoskar RR**, Shah SJ, Shenoy SG. Evaluation of anti-inflammatory property of curcumin (diferuloyl methane) in patients with postoperative inflammation. *Int J Clin Pharmacol Ther Toxicol* 1986; **24**: 651-654
20 **Eamlamnam K**, Patumraj S, Visedopas N, Thong-Ngam D. Effects of Aloe vera and sucralfate on gastric microcirculatory changes, cytokine levels and gastric ulcer healing in rats. *World J Gastroenterol* 2006; **12**: 2034-2039
21 **Dixon MF**, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol* 1996; **20**: 1161-1181
22 **Alican I**, Coşkun T, Corak A, Yeğen BC, Oktay S, Kurtel H. Role of neutrophils in indomethacin-induced gastric mucosal lesions in rats. *Inflamm Res* 1995; **44**: 164-168
23 **Campbell EJ**, Senior RM, McDonald JA, Cox DL. Proteolysis by neutrophils. Relative importance of cell-substrate contact and oxidative inactivation of proteinase inhibitors in vitro. *J Clin Invest* 1982; **70**: 845-852
24 **Weiss SJ**, LoBuglio AF. An oxygen-dependent mechanism of neutrophil-mediated cytotoxicity. *Blood* 1980; **55**: 1020-1024
25 **Zimmerman BJ**, Granger DN. Reperfusion-induced leukocyte infiltration: role of elastase. *Am J Physiol* 1990; **259**: H390-H394
26 **Lazzaroni M**, Bianchi Porro G. Gastrointestinal side-effects of traditional non-steroidal anti-inflammatory drugs and new formulations. *Aliment Pharmacol Ther* 2004; **20** Suppl 2: 48-58
27 **Konturek PC**, Duda A, Brzozowski T, Konturek SJ, Kwiecien S, Drozdowicz D, Pajdo R, Meixner H, Hahn EG. Activation of genes for superoxide dismutase, interleukin-1beta, tumor necrosis factor-alpha, and intercellular adhesion molecule-1 during healing of ischemia-reperfusion-induced gastric injury. *Scand J Gastroenterol* 2000; **35**: 452-463
28 **Kast RE**. Tumor necrosis factor has positive and negative self regulatory feed back cycles centered around cAMP. *Int J Immunopharmacol* 2000; **22**: 1001-1006
29 **Jainu M**, Devi CS. Gastroprotective action of Cissus quadrangularis extract against NSAID induced gastric ulcer: role of proinflammatory cytokines and oxidative damage. *Chem Biol Interact* 2006; **161**: 262-270
30 **Chattopadhyay I**, Bandyopadhyay U, Biswas K, Maity P, Banerjee RK. Indomethacin inactivates gastric peroxidase to induce reactive-oxygen-mediated gastric mucosal injury and curcumin protects it by preventing peroxidase inactivation and scavenging reactive oxygen. *Free Radic Biol Med* 2006; **40**: 1397-1408
31 **Singh S**, Aggarwal BB. Activation of transcription factor NF-kappa B is suppressed by curcumin (diferuloylmethane) [corrected]. *J Biol Chem* 1995; **270**: 24995-25000
32 **Bierhaus A**, Zhang Y, Quehenberger P, Luther T, Haase M, Müller M, Mackman N, Ziegler R, Nawroth PP. The dietary pigment curcumin reduces endothelial tissue factor gene expression by inhibiting binding of AP-1 to the DNA and activation of NF-kappa B. *Thromb Haemost* 1997; **77**: 772-782
33 **Kuner P**, Schubenel R, Hertel C. Beta-amyloid binds to p57NTR and activates NFkappaB in human neuroblastoma cells. *J Neurosci Res* 1998; **54**: 798-804
34 **Pendurthi UR**, Williams JT, Rao LV. Inhibition of tissue factor gene activation in cultured endothelial cells by curcumin. Suppression of activation of transcription factors Egr-1, AP-1, and NF-kappa B. *Arterioscler Thromb Vasc Biol* 1997; **17**: 3406-3413
35 **Jobin C**, Bradham CA, Russo MP, Juma B, Narula AS, Brenner DA, Sartor RB. Curcumin blocks cytokine-mediated NF-kappa B activation and proinflammatory gene expression by inhibiting inhibitory factor I-kappa B kinase activity. *J Immunol* 1999; **163**: 3474-3483
36 **Plummer SM**, Holloway KA, Manson MM, Munks RJ, Kaptein A, Farrow S, Howells L. Inhibition of cyclo-oxygenase 2 expression in colon cells by the chemopreventive agent curcumin involves inhibition of NF-kappaB activation via the NIK/IKK signalling complex. *Oncogene* 1999; **18**: 6013-6020
37 **Sintara K**, Thong-Ngam D, Patumraj S, Klaikeaw N, Chatsuwan T. Curcumin suppresses gastric NF-kappaB activation and macromolecular leakage in Helicobacter pylori-infected rats. *World J Gastroenterol* 2010; **16**: 4039-4046

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ORIGINAL ARTICLE

HBx activates FasL and mediates HepG2 cell apoptosis through MLK3-MKK7-JNKs signal module

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Abstract

AIM: To investigate the possible mechanism by which hepatitis B virus X protein (HBx) mediates apoptosis of HepG2 cells.

METHODS: HBx expression vector pcDNA3.1-X was transfected into HepG2 cells to establish an HBx high-expression cellular model as pcDNA3.1-X transfected group. The pcDNA3.1-X and pSilencer3.1-shHBX (HBx antagonist) were cotransfected into HepG2 cells to establish an HBx low-expression model as RNAi group. Untransfected HepG2 cells and HepG2 cells transfected with negative control plasmid were used as controls. Apoptosis rate, the expression of Fas/FasL signaling pathway-related proteins and the phosphorylation levels of MLK3, MKK7 and JNKs, which are upstream molecules of death receptor pathways and belong to the family of mitogen-activated protein kinases (MAPKs), were measured in each group.

RESULTS: Compared with HepG2 cell group and RNAi group, apoptosis rate, the expression of Fas and FasL proteins, and the activation of MLK3, MKK7 and JNKs were increased in the pcDNA3.1-X transfected group. The activation of JNKs and expression of FasL protein were inhibited in the pcDNA3.1-X transfected group when treated with a known JNK inhibitor, SP600125. When authors treated pcDNA3.1-X transfected group with K252a, a known MLK3 inhibitor, the activation of MLK3, MKK7 and JNKs as well as expression of FasL protein was inhibited. Furthermore, cell apoptosis rate was also significantly declined in the presence of K252a in the pcDNA3.1-X transfected group.

CONCLUSION: HBx can induce HepG2 cell apoptosis *via* a novel active MLK3-MKK7-JNKs signaling module to upregulate FasL protein expression.

Key words: Hepatitis B virus X protein; MLK3; FasL; HepG2 cell; Apoptosis

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INTRODUCTION

Hepatitis B virus (HBV) is one of the major pathogenic causes of primary hepatocellular carcinoma (HCC),

among which, HBV X gene is considered as a key gene that plays a critical role in the occurrence and progression of HBV-related HCC[1]. It has been proposed that HBx is a cellular transactivator that may indirectly stimulate a variety of viral and host gene promoters by interacting with transcription factors, including AP-1, ATF/CREB, ERCC, and is involved in several signal transduction pathways, including mitogen-activated protein kinase, Ras-Raf-mitogen-activated protein kinase, and JAK/STAT signaling pathways, therefore HBx affects several cellular processes, such as proliferation and differentiation[2-4]. In contrast to its proliferative effects, HBx also participates in the apoptotic destruction of liver cells during the virus infection. Several mechanisms might be involved in this process: (1) HBx induces cell apoptosis on its own or sensitizes cells to apoptotic stimuli such as tumor necrosis factor α (TNF-α) or UV irradiation[5-8]; (2) HBx increases expression of IL-18 and enhances transcription activity of Egr-2 and Egr-3, which up-regulates FasL expression and induces the apoptosis of hepatic cells by the death receptor pathway[9,10]; (3) HBx may directly not only target to mitochondria to enhance translocation of Bax to mitochondria but also interact with the mitochondrial protein voltage-dependent anion channel (HVDAC3) to induce cell death by causing loss of mitochondrial membrane potential; and (4) HBx also interacts with heat shock protein 60 which is also localized in mitochondria to enhance HBx-mediated apoptosis[11-13].

Cell death signals from the extracellular environment or internal sensors for the cellular response are major constituents of apoptotic machinery. Cell surface death receptors that transmit cell death signals are activated by specific death ligands. It is demonstrated that Fas is one of the best-characterized death receptors. Upon binding of FasL onto Fas, apoptotic signals are subsequently transmitted *via* death adaptor molecule FADD which can mediate the activation of caspase 8, and active caspase 8 can proteolytically activate downstream effector caspases, such as caspase 3, to trigger apoptosis[14,15]. It is reported that liver cell apoptosis is mediated by Fas[16-18]. Understanding the molecular mechanism responsible for the regulation of Fas and FasL may aid in developing novel therapeutic strategies for HCC.

The mixed lineage kinases (MLKs) are a family of serine/threonine protein kinases that function in a phosphorelay module to control the activity of specific mitogen-activated protein kinases (MAPKs). The family includes three subgroups: MLKs (mixed lineage kinases, including MLK1-4), dual leucine zipper-bearing kinases (DLKs), and Zipper Sterile-a-Motif Kinases (ZAKs). MLKs as mitogen activated protein kinase kinase kinases (MAPKKKs) could activate MKKs, such as MKK4 and/or MKK7, which in turn, activate c-Jun N-terminal kinases (JNKs)[19-21]. Ischemic brain injury studies provide the evidence that the ischemia-stimulating factor can activate MLK3-MKK7-JNKs signaling module to activate death receptor pathway, leading to the neural cell apoptosis[22,23]. It is noteworthy that hepatic cells also express MLK3, MKK7, JNK proteins[24,25]. Therefore, it is of significance to clarify whether the HBx can also activate MLK3-MKK7-JNKs signaling module and induce apoptosis of hepatic cells.

In this study, we demonstrated that HBx induces the apoptosis of hepatic cells depending on activating MLK3-MKK7-JNKs signaling module to upregulate FasL protein expression. On this basis, this study gives a new insight into a better understanding of how HBx mediates apoptosis in hepatocytes, and lays the foundation for further revealing the role of HBx in HBV-related liver oncogenesis and development.

MATERIALS AND METHODS

Reagents

The RPMI 1640 medium, liposome Lipofectamine 2000, and Trizol reagent were obtained from Invitrogen (Carlsbad, CA). Mouse monoclonal anti-HBx antibody was from Chemicon (Temecula, CA). Rabbit polyclonal anti-phospho-MLK3 and rabbit polyclonal anti-phospho-MKK7 were from Cell Signaling Technology (Beverly, MA). Mouse monoclonal anti-phospho-JNKs, rabbit polyclonal anti-Fas, and rabbit polyclonal anti-FasL were from Santa Cruz Biotechnology (Santa Cruz, CA). Mouse monoclonal anti-GAPDH antibody, goat anti-mouse IgG-AP, goat anti-rabbit IgG-AP, BCA Protein Assay Kit, BCIP/NBT Alkaline Phosphatase Color Development Kit, the BeyoECL Plus Western blotting detection System, Caspase3 activity assay kit, Caspase8 activity assay kit, and Hoechst33258 staining solution were from Beyotime Institute of Biotechnology (Jiangsu, China). *In situ* cell death detection kit was from Roche (Mannheim, Germany). Annexin V/PI apoptosis kit was from Biovision (Mountain View, CA). Primers were synthesized by Shanghai Sangon Biological Engineering Technology and Services (Shanghai, China). TIANScript RT Kit was from TIANGEN Biotech (Beijing, China). SP600125 and K252a were obtained from Sigma (St. Louis, MO). Twenty mmol/L stock solution of SP600125 and 20 μmol/L stock solution of K252a were prepared in dimethyl sulfoxide (DMSO) and stored at -20 ℃ in the dark. SP600125 and K252a were prepared freshly for each experiment by serial dilution into 0.01% DMSO in RPMI 1640 medium. All other chemicals and reagents were of analytical grade.

Plasmids construction

Plasmid pcDNA3.1-X containing the full length HBx sequence, was constructed in mammalian expression vector pcDNA3.1 (Invitrogen) as described previously[26].

To construct the expression vector for shRNA targeting HBx, pSilencer3.1-shHBX, two chemically synthesized oligonucleotides encoding HBx specific shRNA with the following sense sequences: 5'-GATCCGGTCTTACATA-AGAGGACTTTCAAGAG AAGTCCTCTTATGTA-AGACCTTTTTTGGAAA-3' and antisense sequences: 5'-AGCTTTTCCAAAAAAGGTCTTACATAAGAG-GACTTCTCTTGAAAGTCCTCTTAT GTA-AGACCG-3' were annealed and cloned into *Bam*H I -

Table 1 Primers for the reverse transcription polymerase chain reaction amplification

Gene	Sense	Antisense	Product length (bp)
HBX	5′-TGTGAAGCTTATGGCTGCTAGGC-3′	5′-TGTGGAATTCTTAGGCAGAGGTG-3′	465
Fas	5′-GTGAACACTGTGACCCTT-3′	5′-TCATTGACACCATTCTTTCG-3′	349
FasL	5'-CTGGGGATGTTTCAGCTCTTC-3′	5'-CTTCACTCCAGAAAGCAGGAC-3'	304
Bax	5′-TTTGCTTCAGGGTTTCATCC-3′	5′-CAGTTGAAGTTGCCGTCAGA-3′	246
BcL-2	5′-GTGGAGGAGCTCTTCAGGGA-3′	5′-AGGCACCCAGGGTGATGCAA-3	304
β-actin	5′-GGCATCGTGATGGACTCCG-3′	5′-GCTGGAAGGTGGACAGCGA-3	607

HBx: Hepatitis B virus X protein.

*Hin*dⅢ sites of the linearized pSilencer3.1-H1-nero vector (Ambion, Austin, TX).

Cell culture and transfection

Human hepatocarcinoma cell line, HepG2 cell, obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China) was cultured in RPMI 1640 medium supplemented with 100 mL/L fetal bovine serum, 2 mmol/mL L-glutamine, 100 μg/mL streptomycin and 100 units/mL penicillin at 37 ℃ in 5% CO_2. When the cell fusion rate reached 80%, HepG2 cells were transfected with negative control plasmid pcDNA3.1, pcDNA3.1-X, cotransfected pcDNA3.1-X with either pSilencer3.1-shHBX or pSilencer3.1-H1 in a ratio of 1:3, in the presence of the liposome Lipofectamine 2000 according to the manufacturer's instructions.

Reverse transcription polymerase chain reaction analysis

The total RNA of HepG2 cells transfected with various plasmids was prepared with Trizol reagent according to the manufacturer's instructions. The reverse transcription was performed with TIANScript RT Kit. The specific primers used are shown in Table 1, the amplification condition was 94 ℃ for 45 s, 58 ℃ (55 ℃-60 ℃) for 35s, 72 ℃ for 1 min for 35 cycles and a final extension at 72 ℃ for 5 min each. The PCR products were subjected to electrophoresis in 1% agarose gel and visualized by ethidium bromide staining.

Western blotting analysis

For protein extracts, cells were lysed using cell lysis buffer [20 mmol/L Tris pH 7.5, 150 mmol/L NaCl, 1% TritonX-100, 2.5 mmol/L sodium pyrophosphate, 1 mmol/L EDTA, 1% Na_3VO_4, 0.5 μg/mL leupeptin and other phosphatase inhibitors, 1 mmol/L phenylmethanesulfonyl fluoride (PMSF)]. The lysates were collected, and centrifuged at 10 000 × *g* at 4 ℃ for 5 min. The bicinchoninic acid (BCA) Protein Assay Kit was used to measure the protein concentrations. Total protein of 100 μg of each above lysate was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes, which were blocked with 3% bovine serum albumin (BSA) in tris buffered saline (TBS) containing 0.01% Tween-20 for 3 h at room temperature, and then incubated with specific primary antibodies: mouse monoclonal anti-HBx antibody (1:250), rabbit polyclonal anti-phospho-MLK3 (1:500), rabbit polyclonal anti-phospho-MKK7(1:500), mouse monoclonal anti-phospho-JNKs (1:400), rabbit polyclonal anti-Fas (1:500), rabbit polyclonal anti-FasL (1:500), and mouse monoclonal anti-GAPDH antibody (1:500), respectively, overnight at 4 ℃. The membranes were then incubated with goat anti-mouse IgG-AP (1:500), goat anti-rabbit IgG-AP (1:500), goat anti-mouse IgG-HRP (1:2000) and goat anti-rabbit IgG-HRP (1:2000) for 2 h at room temperature separately. The labeled bands were detected with NBT/BCIP Alkaline Phosphatase Color Development Kit or the BeyoECL Plus Western blotting detection system.

Caspase activity assay

The enzyme activities of caspase3, caspase8 and caspase9 were quantified using caspase3, caspase8 and caspase9 activity assay kit, respectively. Adherent and floating cells were collected and lysed in caspase lysis buffer. Caspase3 enzyme activity in 30 μg cell lysate was measured by cleavage of Ac-DEVD-pNA colorimetric substrate. Caspase8 enzyme activity in 30 μg cell lysate was measured by cleavage of Ac-IETD-pNA colorimetric substrate. Caspase9 enzyme activity in 30 μg cell lysate was measured by cleavage of Ac-LEHD-pNA colorimetric substrate. The absorbance at $A_{405\ nm}$ was quantified in a microtiter plate reader after incubated at 37 ℃ for 2 h.

Apoptosis analysis

Cells were adjusted to a density of 2×10^5 cells/mL, added to 24-well plates in 0.5 mL each well. After transfection and incubation for 72 h, cell apoptosis was analyzed by three methods: (1) terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) staining: cell apoptosis was analyzed using the *in situ* cell death detection kit, according to the manufacturer's protocol. The number of TUNEL-positive cells was divided by the total number of cells to determine the ratio of TUNEL-positive cells. Five optical fields, about 200 cells were selected randomly and analyzed; (2) Hoechst 33258 staining: cells were fixed with 4% paraformaldehyde, washed twice with phosphate-buffered saline (PBS) and stained with Hoechst 33258 staining solution according to the manufacturer's instructions. The morphologic changes of apoptotic cells, including reduction in volume and nuclear chromatin condensation, were observed under a fluorescence microscope. Five optical fields containing about 200 cells were selected randomly and analyzed; and (3) flow

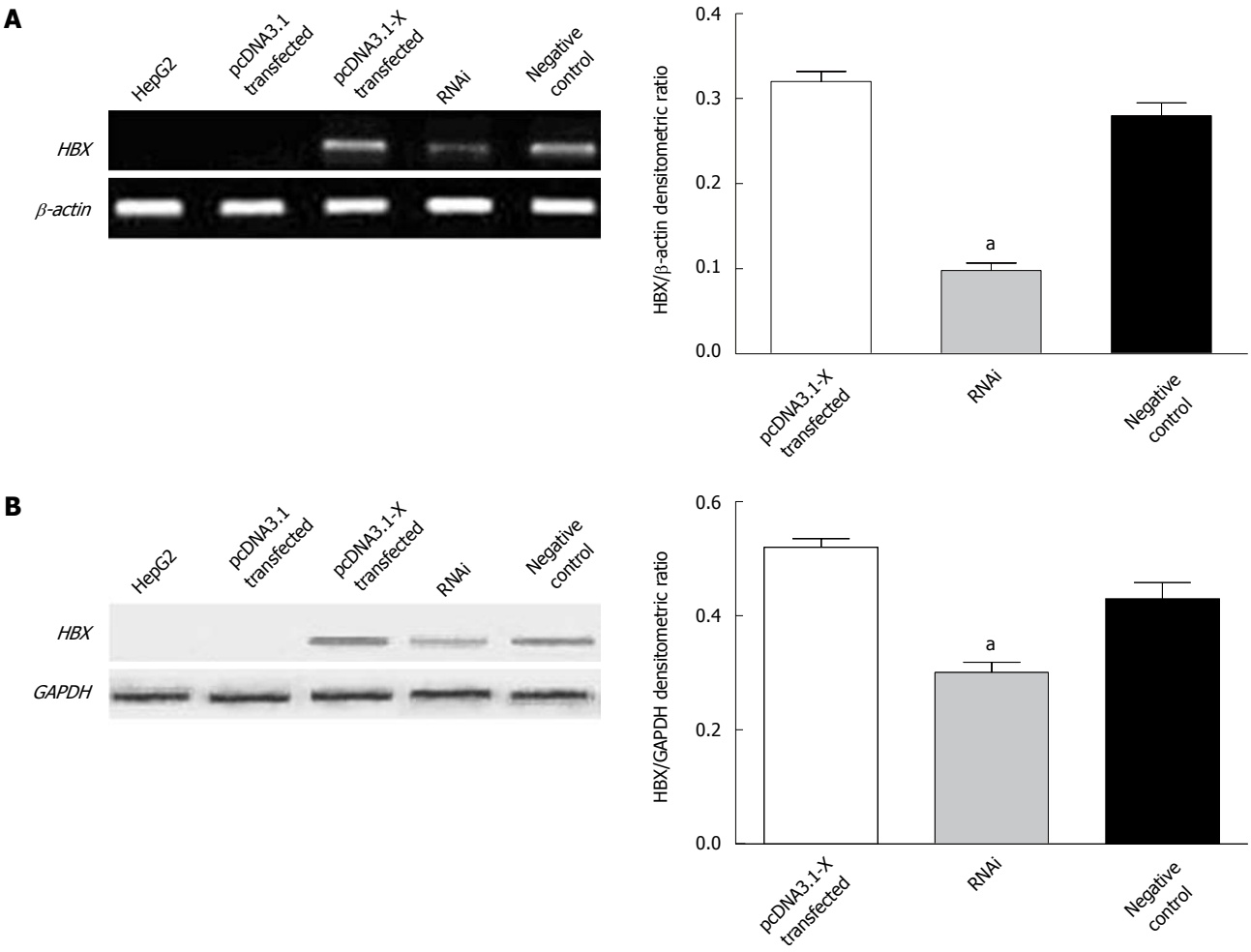

Figure 1 Detection of hepatitis B virus X protein expression in transfected HepG2 cells. HepG2 cells were transfected with pcDNA3.1-X plasmids or cotransfected with pcDNA3.1-X and pSilencer3.1-shHBX plasmids. Forty-eight hours later, the expression of hepatitis B virus X protein (HBx) in HepG2 cells was determined by reverse transcription polymerase chain reaction (A) and Western blotting analysis (B). HepG2 group was not transfected with any plasmids. pcDNA3.1 transfected group was transfected with plasmid pcDNA3.1; pcDNA3.1-X transfected group was transfected with pcDNA3.1-X; RNAi group was cotransfected with pcDNA3.1-X and pSilencer3.1-shHBX in a ratio of 1:3; negative control group was cotransfected with pcDNA3.1-X and negative control plasmid pSilencer3.1-H1 in a ratio of 1:3. Data are expressed as mean ± SD (n = 3), $^aP < 0.05$ *vs* pcDNA3.1-X transfected group.

cytometry: cell apoptosis was evaluated by double staining with fluorescein isothiocyanate (FITC)-conjugated Annexin V and propidium iodide (PI) using annexin V/PI apoptosis kit. Cells were washed with PBS twice and stained with Annexin V and PI for 5 min at room temperature in the dark. The level of apoptosis was determined by measuring the fluorescence of the cells with a flow cytometer (Becton-Dickinson, San Diego, CA).

Statistical analysis

All experiments were performed three times. Semiquantitative analysis of the bands was performed with the Image J analysis software (Version 1.30v, Wayne Rasband, NIH, United States). The data were presented in mean ± SD and analyzed by one-way ANOVA (SPSS version 13.0). $P < 0.05$ was considered statistically significant.

RESULTS

Expression of hepatitis B virus X protein in HepG2 cells

To investigate the potential apoptotic ability of HBx, HBx expressing plasmid pcDNA3.1-X was transiently transfected into a human HCC cell line, HepG2. RT-PCR and Western blotting analysis demonstrated that HepG2 cells transfected with pcDNA3.1-X could steadily express HBx. To further identify the function of HBx upon apoptosis of HepG2, the expression vector for shRNA targeting HBx named pSilencer3.1-shHBX was constructed. When the shRNA expression plasmid was transfected to HepG2 cells in combination with pcDNA3.1-X, the expression of HBx was specifically inhibited by shRNA against HBx, while universal negative control plasmid pSilencer3.1-H1 did not display any effect on HBx expression (Figure 1).

Effects of hepatitis B virus X protein on induction of apoptosis in HepG2 cells

To investigate the roles of HBx in cell apoptosis, TUNEL assay was used to detect the presence of DNA strand breaks. The *in situ* TUNEL staining showed that the TUNEL-negative cells appeared with blue nucleus and TUNEL-positive cells with yellow nucleus. To fur-

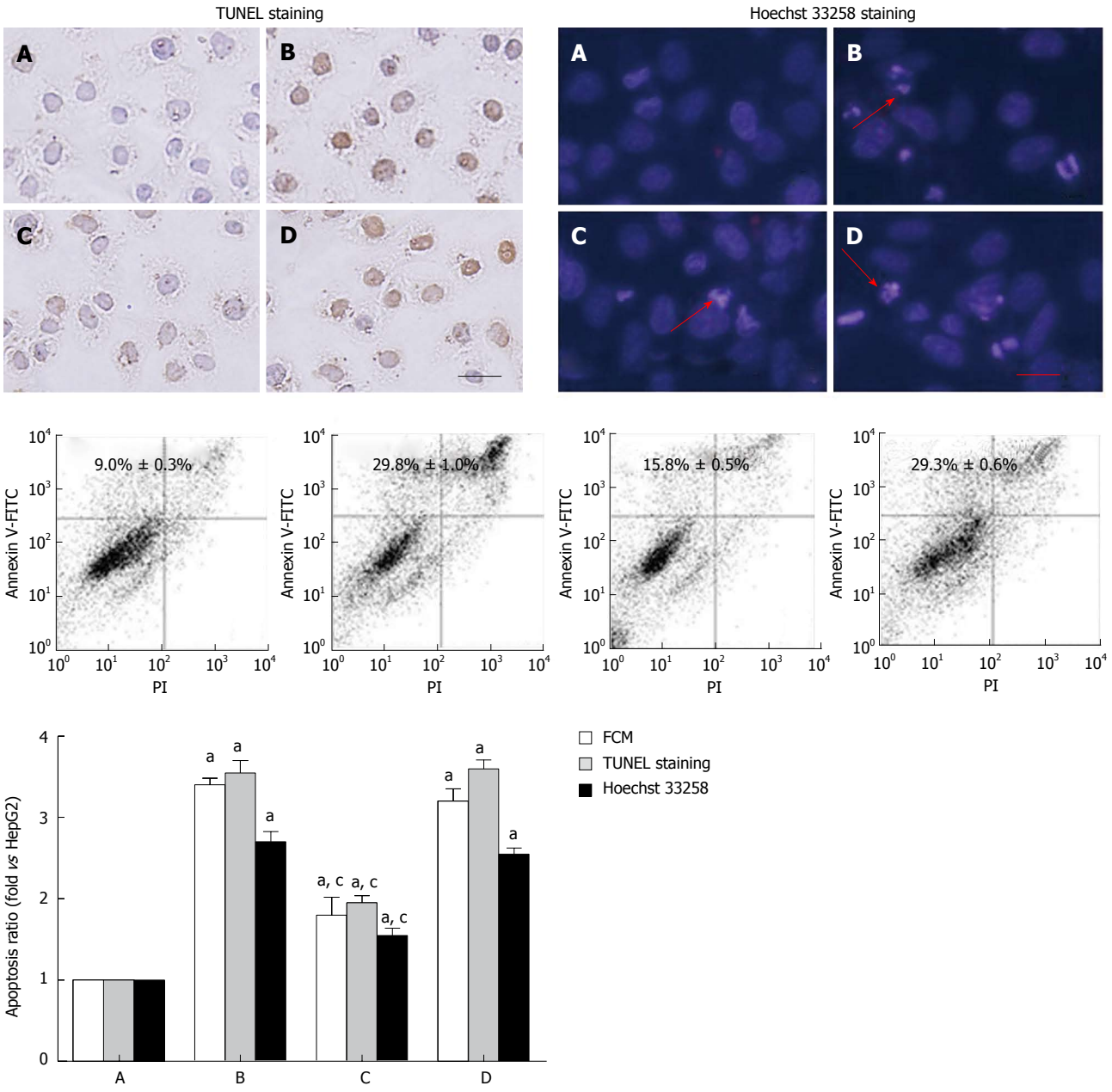

Figure 2 Apoptosis of HepG2 cells induced by hepatitis B virus X protein. HepG2 cells were cotransfected with pcDNA3.1-X and pSilencer3.1-shHBX as RNAi group, and HepG2 cells were cotransfected with pcDNA3.1-X and pSilencer3.1-H1 plasmids as negative control. Cells were examined by TUNEL, Hoechst 33258 staining and flow cytometry as described in Materials and Methods. A: HepG2 group; B: pcDNA3.1-X transfected group; C: RNAi group; D: Negative control group. Data was expressed as mean ± SD (n = 3), $^{a}P < 0.05$ *vs* the HepG2 group; $^{c}P < 0.05$ *vs* pcDNA3.1-X transfected group and negative control group. Scale bar value: 5 μm. Red arrows indicate apoptotic nuclei. Apoptosis ratio= (apoptotic cells/total cells) × 100%. TUNEL: Terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling.

ther confirm the occurrence of apoptosis, the apoptosis of HepG2 cells and HepG2 transfected with HBx were detected by Hoechst 33258 staining and flow cytometry (FCM). In Hoechst 33258 staining, reduced cell sizes and increased nuclear chromatin condensation were detected in the apoptotic cells. As it is shown in Figure 2, when compared with HepG2 cells group, the apoptosis rate of pcDNA3.1-X transfected group and negative control group was increased, while, in contrast, that of RNAi group was decreased. It indicated that the high expression of HBx could promote the apoptosis of HepG2 cells, and inhibiting the expression of HBx protein could reduce the apoptosis rate of HepG2 cells.

Hepatitis B virus X protein-induced apoptosis was attributed to the upregulation of Fas/FasL signaling pathway-related proteins

To determine which signaling pathway was involved in cell apoptosis by HBx, expression of Fas, FasL and apoptotic regulators Bax, Bcl-2 in HBx-transfected HepG2 cells were examined by RT-PCR and Western blotting analysis. The results showed that the Fas, FasL, Bax mRNA and protein expression were increased induced by HBx, while the Bcl-2 mRNA and protein were decreased

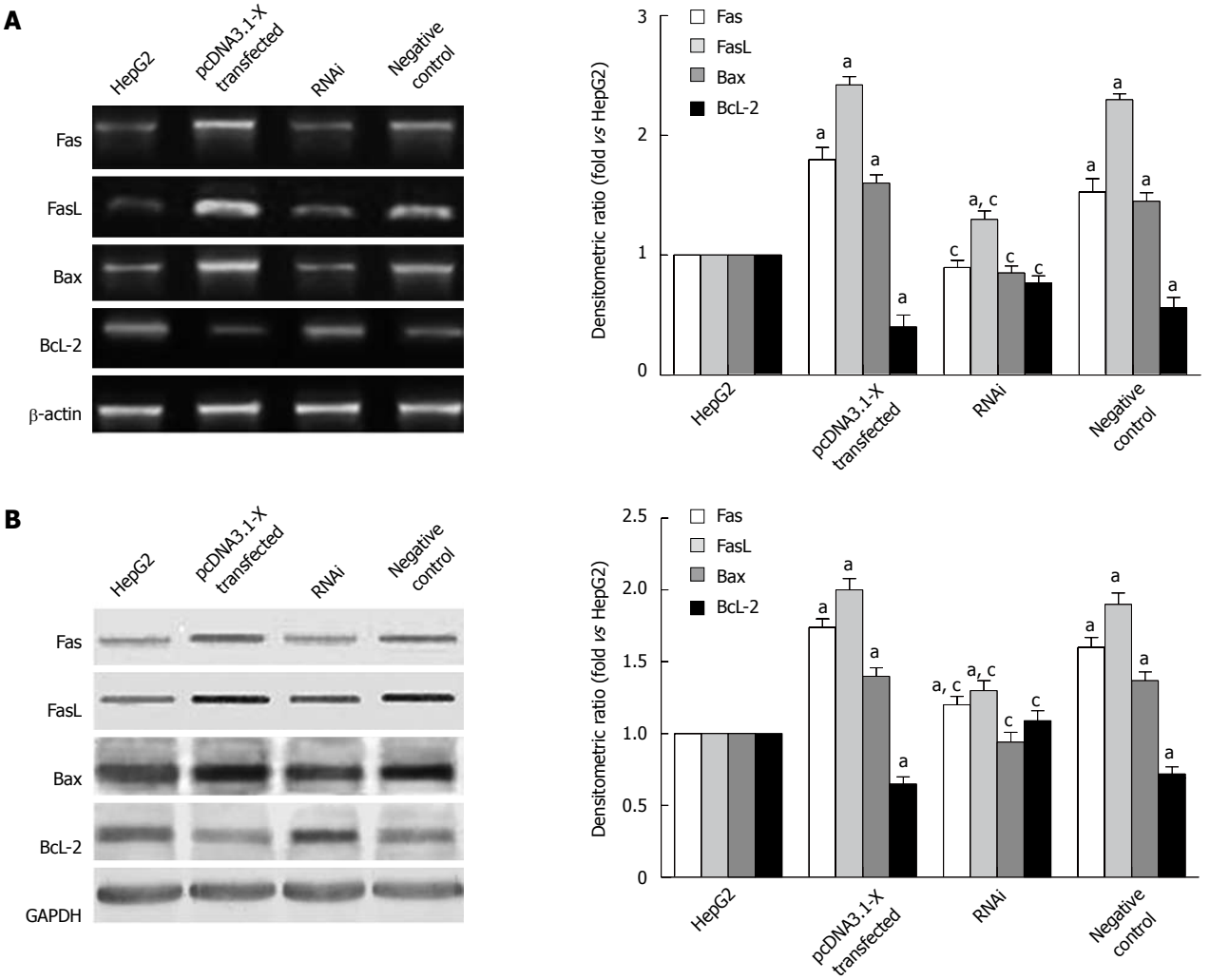

Figure 3 Expression of Fas, FasL, Bax, Bcl-2 mRNA and protein in HepG2 cells induced by hepatitis B virus X protein. Transfection of HepG2 cells is described in Figure 2. Forty-eight hours after transfection, the mRNA (A) and protein (B) expression levels of Bax, Bcl-2, Fas and FasL were determined by RT-PCR and Western blotting analysis. Data are expressed as mean ± SD (n = 3), $^{a}P < 0.05$ *vs* the HepG2 group; $^{c}P < 0.05$ *vs* pcDNA3.1-X transfected group and negative control group.

after transfection with HBx in HepG2 cells for 48 h (Figure 3). When HepG2 cells were cotransfected with pcDNA3.1-X and pSilencer3.1-shHBX for 48 h, the levels of Fas, FasL, Bax mRNA and protein were decreased. However, Bcl-2 mRNA and protein were increased after cotransfection with pcDNA3.1-X and pSilencer3.1-shHBX in HepG2 cells.

In order to determine the dynamic effect of HBx on the Fas/FasL signaling pathway, we detected the gene and protein expression of Fas and FasL at 24 h, 48 h, and 72 h after transfection with HBx, respectively, and found that the Fas and FasL mRNA expression was increased by HBx in a time-dependent manner (Figure 4A). In parallel, the levels of Fas and FasL proteins in pcDNA3.1-X transfected cells were increased by HBx in a similar manner (Figure 4B).

To further determine the effect of HBx on the Fas/FasL signaling pathway, enzyme activity of caspase8, caspase9 and caspase3 were also detected by spectrophotometric test. The results showed that the activation of caspase8, caspase9 and caspase3 was increased in HBx-transfected HepG2 cells (Figure 5).

It could be concluded that HBx could mediate cell apoptosis by upregulating Fas/FasL signaling pathway-related protein expression to activate the Fas/FasL signaling pathway.

Hepatitis B virus X protein upregulates FasL protein expression by activating MLK3-MKK7-JNKs signal module and induces apoptosis

The upstream mechanisms of HBx on expression of Fas/FasL signaling pathway-related proteins were further investigated. Previous studies showed that MLK3, MKK7 and JNKs which belong to MAPK signaling pathways, could form MLK3-MKK7-JNKs signaling module and the activation of the signaling module could upregulate the expression of Fas/FasL signaling pathway-related protein, leading to cell apoptosis[22,23]. To find out whether the upregulation of Fas/FasL signaling pathway-related proteins induced by HBx was dependent on activation of MLK3-MKK7-JNKs signaling module, the phosphorylation levels of MLK3, MKK7 and JNKs

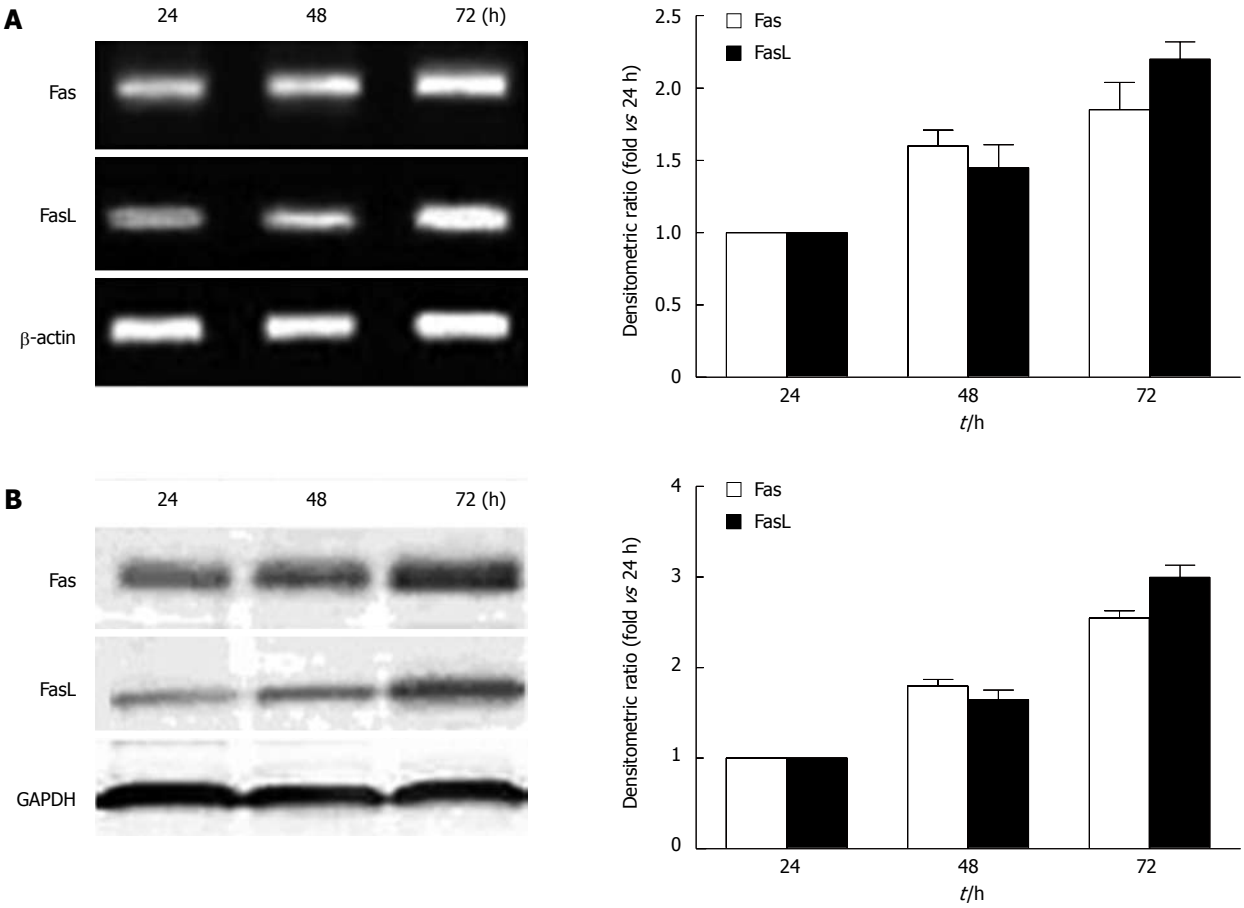

Figure 4 Upregulation of Fas/FasL signaling pathway-related protein expression by hepatitis B virus X protein in a time-dependent manner. HepG2 cells were transfected with pcDNA3.1-X and incubated for various time periods as indicated. The levels of Fas, FasL mRNA (A) and proteins (B) were determined by reverse transcription polymerase chain reaction and Western blotting analysis. Data are expressed as mean ± SD (n = 3).

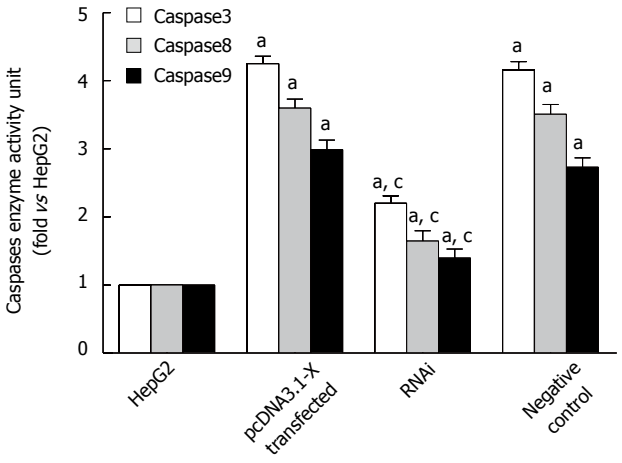

Figure 5 Detection of activated caspases in HepG2 cells transfected with hepatitis B virus X protein. Transfection of HepG2 cells is described in Figure 2. Forty-eight hours after transfection, the enzyme activity of caspase3, caspase8 and caspase9 was analyzed by spectrophotometric test. Data are expressed as mean ± SD (n = 3), $^{a}P < 0.05$ *vs* the HepG2 group; $^{c}P < 0.05$ *vs* pcDNA3.1-X transfected group and negative control group.

were detected. The phosphorylation levels of MLK3, MKK7 and JNKs proteins in pcDNA3.1-X transfected group were increased remarkably compared with the HepG2 cells. When silencing HBx protein expressed by pSilencer3.1-shHBX, phosphorylation of MLK3, MKK7 and JNKs was inhibited (Figure 6). The changing tendency of expression level of FasL protein was consistent with phosphorylation levels of MLK3, MKK7 and JNKs proteins. When we treated HepG2 cells transfected with HBx with a known JNK inhibitor, SP600125, the activation of JNKs and expression of FasL protein were inhibited (Figure 7A). Furthermore, the phosphorylation levels of MLK3, MKK7 and JNKs and the protein expression of FasL also concomitantly decreased in pcDNA3.1-X transfected group when treated with K252a, a known MLK3 inhibitor[27,28] (Figure 7B). The results demonstrated that HBx could activate MLK3-MKK7-JNKs signal module and upregulate Fas/FasL death receptor pathway-related protein expression.

To determine the relationship between activation of MLK3-MKK7-JNKs signal module and HBx-stimulated cell apoptosis, the apoptosis rate of the pcDNA3.1-X transfected group treated with K252a or not was determined by flow cytometry. As expected, apoptosis rate of the HBx-transfected HepG2 cells was suppressed in the presence of the MLK3 inhibitor (Figure 8), indicating that HBx could activate the MLK3-MKK7-JNKs signaling modular and its downstream death receptor pathway, and

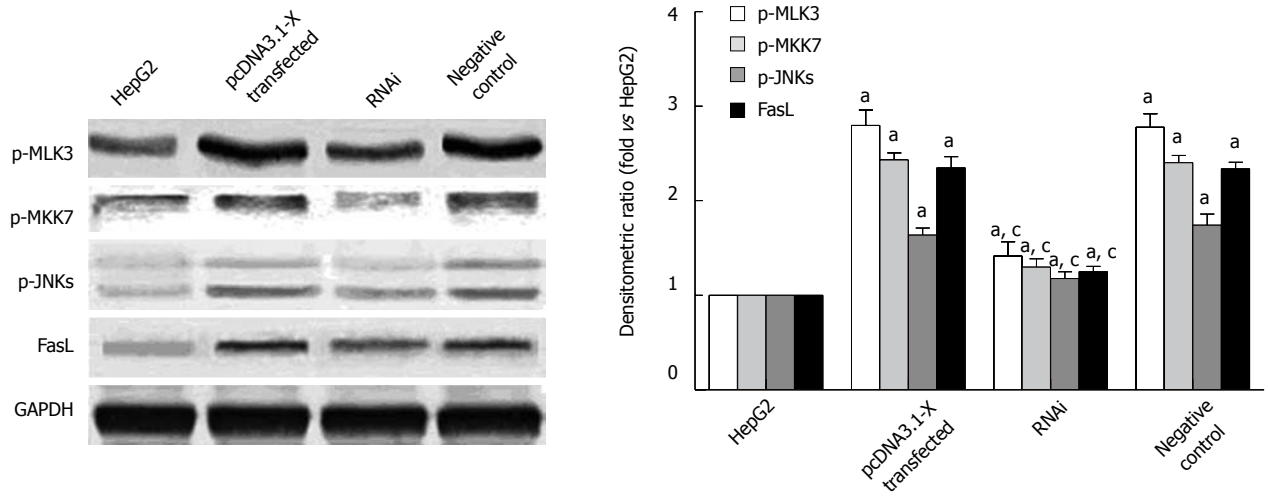

Figure 6 Activation of MLK3-MKK7-JNKs signaling module in hepatitis B virus X protein-transfected HepG2 cells. Transfection of HepG2 cells is described in Figure 2. Forty-eight hours after transfection, the cells lysates were detected by Western blotting analysis. Data are expressed as mean ± SD (n = 3), $^{a}P < 0.05$ *vs* the HepG2 group; $^{c}P < 0.05$ *vs* pcDNA3.1-X transfected group and negative control group.

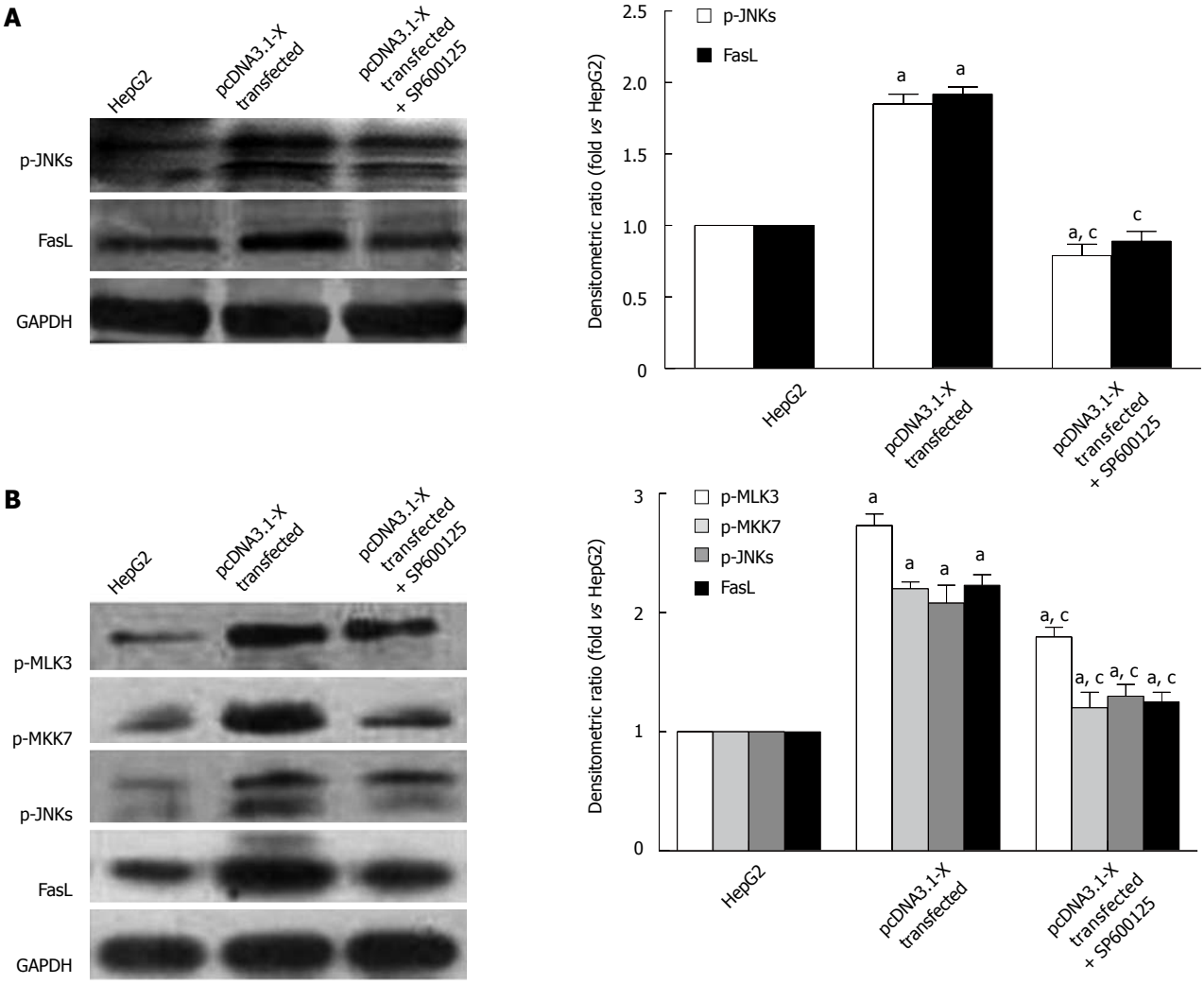

Figure 7 Activation of MLK3-MKK7-JNKs signaling module on FasL expression mediated by hepatitis B virus X protein. A: HepG2 cells were cultured in 0.01% dimethyl sulfoxide (DMSO) in the absence or presence of 20 μmol/L SP600125 after transfection with pcDNA3.1-X and incubated for 24 h; B: HepG2 cells were cultured in 0.01% DMSO in the absence or presence of 300 nmol/L K252a after transfection with pcDNA3.1-X and incubated for 24 h. Cell lysates were prepared and electrophoresed in SDS-PAGE and subsequently performed by Western blotting analysis. Data are expressed as mean ± SD (n = 3), $^{a}P < 0.05$ *vs* the HepG2 group; $^{c}P < 0.05$ *vs* pcDNA3.1-X transfected group.

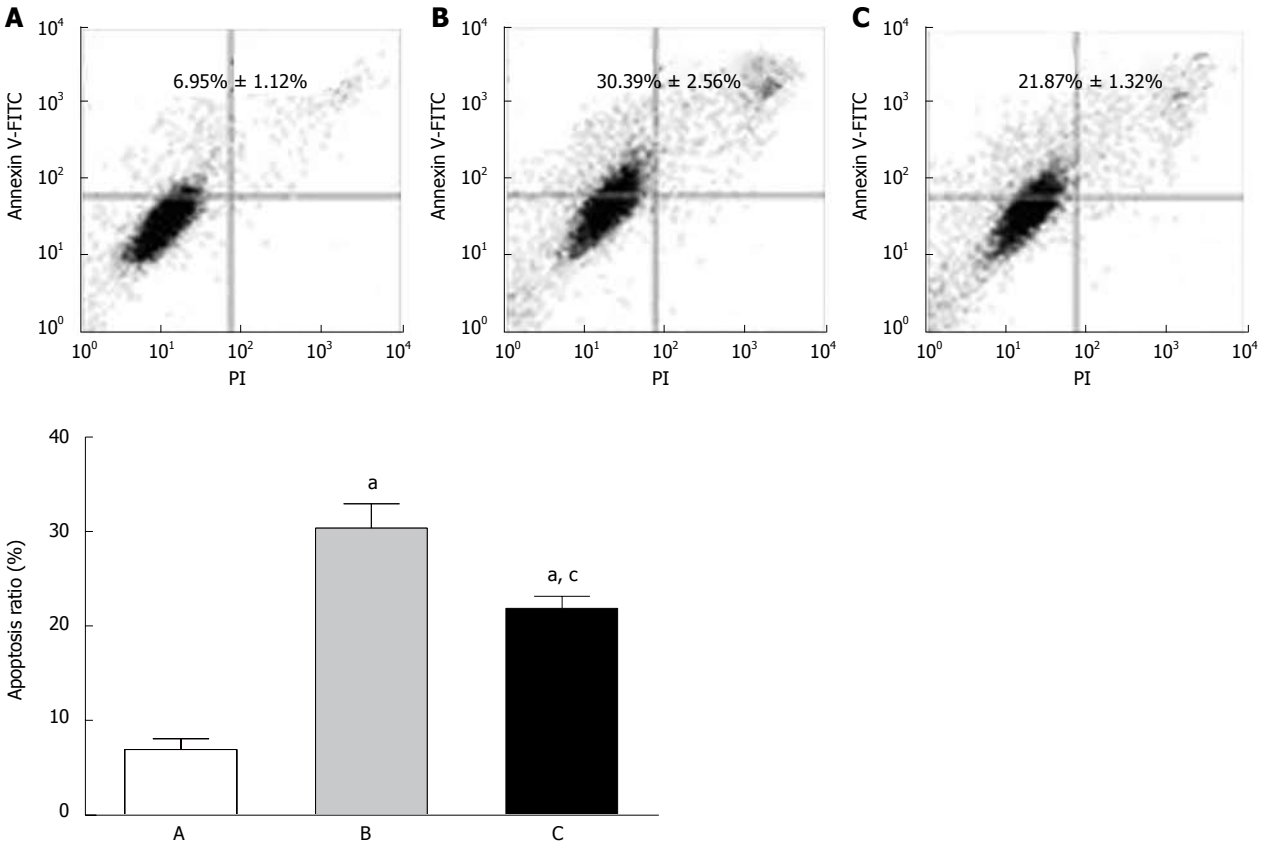

Figure 8 Inhibition of HBx-induced cell apoptosis by K252a. HepG2 cells were cultured in 0.01% dimethyl sulfoxide in the absence or presence of 300 nmol/L K252a after transfection with pcDNA3.1-X and incubated for 24 h, and the cell apoptosis was examined by flow cytometry. A: HepG2 group; B: pcDNA3.1-X transfected group; C: pcDNA3.1-X transfected + K252a group. Data are expressed as mean ± SD (n = 3), $^{a}P < 0.05$ *vs* the HepG2 group; $^{c}P < 0.05$ *vs* pcDNA3.1-X transfected group.

induce the apoptosis of the hepatocarcinoma cell line.

DISCUSSION

HBx protein has been reported to be either a promoter or an inhibitor of cell apoptosis[11,29-31]. The dual activity of HBx protein on cell apoptosis suggests that the expression of HBx gene and its physiological role depend on cellular environments and infection stage[30,32-33]. Naturally, HBx shows extremely low levels of expression during the early stage of HBV infection, which may contribute to the activation of transcription and virus replication[33,34]. With the development of chronic HBV infection, expression of HBx protein increased and activated apoptosis to contribute to virus spread and the progression of chronic hepatitis and HCC[32,35,36]. It was reported that HBx could induce the apoptosis of hepatic cells by the death receptor pathway and the mitochondrial pathway. However, the question how HBx activates the death receptor pathway to induce the apoptosis of hepatic cells remains unclear. The further elucidation of signaling pathways that regulate apoptosis by HBx would help us understand the effectiveness of HBX in the development of HCC.

In this study, the regulation mechanism of apoptosis by HBx in HepG2 cells was investigated. First, the ability of HBx to induce apoptosis using transient transfection of HBx was tested. As a result, after transfected with HBx, HepG2 cells exhibited more conspicuous apoptotic nuclear condensation and a higher level of cell death, which is in agreement with the previous reports that HBx could induce cell apoptosis on its own[5-6,37,38]. Second, the expression level of the Fas/FasL signaling pathway-related proteins was examined in HepG2 cells. The expression of Fas/FasL signaling pathway-related proteins were remarkably upregulated in pcDNA3.1-X transfected group. RNA interference targeting HBx in HepG2 cells could reduce the cell apoptosis and down-upregulate the Fas/FasL signaling pathway-related proteins at the same time. Based on the above results, it was reasonable to conclude that HBx is able to induce apoptosis by upregulating Fas/FasL signaling pathway-related protein expression in HepG2 cells, which was similar to the previous report that HBx played a role in inducing apoptosis of hepatocyte *via* Fas/FasL system[39,40].

Recent studies have found that the MAPKs were involved in the signal transduction for apoptosis. The animal model experiments for cerebral ischemia demonstrated that MLK3-MKK7-JNKs signaling modules, a member of the MAPK family, could activate its downstream Fas/FasL signaling pathway and induce the apoptosis of nerve cells[22-23]. In addition, it was reported that hepatic cells

could also express MLK3, MKK7 and JNKs[24-25]. We supposed HBx protein could act as the stressor to activate the signal module of MLK3-MKK7-JNKs, and to induce the apoptosis of the hepatic cells.

On the basis of this hypothesis, the phosphorylation levels of MLK3, MKK7 and JNKs were detected in all groups. It was found that the phosphorylation levels of MLK3, MKK7 and JNKs were increased obviously in the pcDNA3.1-X group, indicating that the HBx might increase the phosphorylation levels of these proteins. When we treated pcDNA3.1-X group with SP600125, the activation of JNKs and expression of FasL were significantly inhibited. Moreover, when pcDNA3.1-X group was treated with K252a, the expression levels of p-MLK3, p-MKK7, p-JNKs and FasL were also significantly decreased, and then cell apoptosis rate obviously declined, suggesting that the HBx protein could act as a stressor and activate the MLK3-MKK7-JNKs signaling module and its downstream Fas/FasL death receptor pathway to induce the apoptosis of HepG2 cells. This would provide us a new research target and a novel concept for blocking the HBx-induced apoptosis process.

The ratio of Bax and Bcl-2 could change the mitochondrial membrane potential and result in the release of cytochrome C, the activation of caspase9, and then further activate caspase3 to induce apoptosis[41,42]. As shown in Figures 3 and 5, HBx up-regulated the expression of Bax, down-regulated the expression of BcL-2, and increased the activation of caspase9 at the same time. This data indicated that the apoptosis induced by HBx also referred to the mitochondrial pathway. Nevertheless, effects of activating the mitochondrial pathway on apoptosis needs to be further investigated.

In conclusion, this study demonstrated that the high expression level of HBx protein could activate MLK3-MKK7-JNKs module and upregulate FasL protein. It has provided a novel insight into the mechanism of HBx-induced heptocarcinoma cell apoptosis.

ACKNOWLEDGMENTS

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COMMENTS

Background

Hepatitis B virus (HBV) infection is one of the most widely spread viral diseases and strongly associated with the development of hepatocellular carcinoma (HCC). However, the mechanism of HBV-mediated HCC development is not clearly elucidated. Although it has been shown that HBx, the protein encoded by the X gene of the HBV genome, could induce cell apoptosis on its own or sensitize cells to apoptotic stimuli, such as tumor necrosis factor α (TNF-α) or UV irradiation, the mechanism of HBx-mediated apoptosis remains controversial.

Research frontiers

HBx, the protein encoded by the X gene of the HBV genome, is a multifunctional regulatory protein and has been implicated in HBV-mediated hepatocarcinogenesis. In this study, the authors further investigated the regulation mechanism of apoptosis mediated by HBx in HepG2 cells.

Innovations and breakthroughs

Previous researches showed that HBx participated in the apoptotic destruction of liver cells during the virus infection. Although some studies found that HBx could mediate apoptosis by the death receptor pathway or affecting mitochondrial physiology, the mechanism of HBx-mediated apoptosis is still not clear. In the present study, the authors showed that HBx could induce HepG2 cells apoptosis *via* a novel active MLK3-MKK7-JNKs signaling module and upregulate Fas/FasL signaling pathway-related protein expression.

Applications

This study provided a new insight into a better understanding of how HBx mediated apoptosis, and lay the foundation for further clarifying the role of HBx in HBV-related liver oncogenesis and development.

Terminology

Apoptosis is a common form of cell death and often referred to as programmed death. The characteristics of apoptosis include activation of cysteine proteases (caspases) and endonucleases, condensation of the nuclear chromatin and cytoplasm, cleavage of the DNA into oligonucleosomal fragments, and segmentation of the dying cell into membrane-bound apoptotic bodies. Fas is one of the best-characterized death receptor. Upon binding of FasL (Fas ligand) onto Fas, apoptotic signals are subsequently transmitted in cytoplasm to trigger apoptosis. The mixed lineage kinase 3 (MLK3) is a member of the mixed lineage kinases (MLKs), a family of serine/threonine protein kinases functioning in a phosphorelay module to control the activity of specific mitogen-activated protein kinases (MAPKs).

Peer review

In this manuscript, the authors demonstrate that hepatitis B virus X protein induced apoptosis through Fas/FasL signaling *via* MLK3-MKK7-JNKs signaling module in HepG2 cells. A series of experiments are well-planned and well-performed and this manuscript is well written. Although interesting, this manuscript could be strengthened if several points were addressed.

REFERENCES

1 **Neuveut C**, Wei Y, Buendia MA. Mechanisms of HBV-related hepatocarcinogenesis. *J Hepatol* 2010; **52**: 594-604
2 **Bouchard MJ**, Schneider RJ. The enigmatic X gene of hepatitis B virus. *J Virol* 2004; **78**: 12725-12734
3 **Tang H**, Oishi N, Kaneko S, Murakami S. Molecular functions and biological roles of hepatitis B virus x protein. *Cancer Sci* 2006; **97**: 977-983
4 **Zhang X**, Zhang H, Ye L. Effects of hepatitis B virus X protein on the development of liver cancer. *J Lab Clin Med* 2006; **147**: 58-66
5 **Miao J**, Chen GG, Chun SY, Lai PP. Hepatitis B virus X protein induces apoptosis in hepatoma cells through inhibiting Bcl-xL expression. *Cancer Lett* 2006; **236**: 115-124
6 **Pollicino T**, Terradillos O, Lecoeur H, Gougeon ML, Buendia MA. Pro-apoptotic effect of the hepatitis B virus X gene. *Biomed Pharmacother* 1998; **52**: 363-368
7 **Kim WH**, Hong F, Jaruga B, Zhang ZS, Fan SJ, Liang TJ, Gao B. Hepatitis B virus X protein sensitizes primary mouse hepatocytes to ethanol- and TNF-alpha-induced apoptosis by a caspase-3-dependent mechanism. *Cell Mol Immunol* 2005; **2**: 40-48
8 **Kim SY**, Kim JK, Kim HJ, Ahn JK. Hepatitis B virus X protein sensitizes UV-induced apoptosis by transcriptional transactivation of Fas ligand gene expression. *IUBMB Life* 2005; **57**: 651-658
9 **Lee MO**, Choi YH, Shin EC, Kang HJ, Kim YM, Jeong SY, Seong JK, Yu DY, Cho H, Park JH, Kim SJ. Hepatitis B virus X protein induced expression of interleukin 18 (IL-18): a potential mechanism for liver injury caused by hepatitis B virus (HBV) infection. *J Hepatol* 2002; **37**: 380-386
10 **Yoo YG**, Lee MO. Hepatitis B virus X protein induces expression of Fas ligand gene through enhancing transcriptional activity of early growth response factor. *J Biol Chem* 2004; **279**: 36242-36249
11 **Kim HJ**, Kim SY, Kim J, Lee H, Choi M, Kim JK, Ahn JK. Hepatitis B virus X protein induces apoptosis by enhancing translocation of Bax to mitochondria. *IUBMB Life* 2008; **60**: 473-480

12 **Rahmani Z**, Huh KW, Lasher R, Siddiqui A. Hepatitis B virus X protein colocalizes to mitochondria with a human voltage-dependent anion channel, HVDAC3, and alters its transmembrane potential. *J Virol* 2000; **74**: 2840-2846

13 **Tanaka Y**, Kanai F, Kawakami T, Tateishi K, Ijichi H, Kawabe T, Arakawa Y, Kawakami T, Nishimura T, Shirakata Y, Koike K, Omata M. Interaction of the hepatitis B virus X protein (HBx) with heat shock protein 60 enhances HBx-mediated apoptosis. *Biochem Biophys Res Commun* 2004; **318**: 461-469

14 **Guicciardi ME**, Gores GJ. Life and death by death receptors. *FASEB J* 2009; **23**: 1625-1637

15 **Strasser A**, Jost PJ, Nagata S. The many roles of FAS receptor signaling in the immune system. *Immunity* 2009; **30**: 180-192

16 **Akazawa Y**, Gores GJ. Death receptor-mediated liver injury. *Semin Liver Dis* 2007; **27**: 327-338

17 **Corazza N**, Jakob S, Schaer C, Frese S, Keogh A, Stroka D, Kassahn D, Torgler R, Mueller C, Schneider P, Brunner T. TRAIL receptor-mediated JNK activation and Bim phosphorylation critically regulate Fas-mediated liver damage and lethality. *J Clin Invest* 2006; **116**: 2493-2499

18 **Malhi H**, Gores GJ. Cellular and molecular mechanisms of liver injury. *Gastroenterology* 2008; **134**: 1641-1654

19 **Gallo KA**, Johnson GL. Mixed-lineage kinase control of JNK and p38 MAPK pathways. *Nat Rev Mol Cell Biol* 2002; **3**: 663-672

20 **Handley ME**, Rasaiyaah J, Chain BM, Katz DR. Mixed lineage kinases (MLKs): a role in dendritic cells, inflammation and immunity? *Int J Exp Pathol* 2007; **88**: 111-126

21 **Jaeschke A**, Davis RJ. Metabolic stress signaling mediated by mixed-lineage kinases. *Mol Cell* 2007; **27**: 498-508

22 **Liu XM**, Pei DS, Guan QH, Sun YF, Wang XT, Zhang QX, Zhang GY. Neuroprotection of Tat-GluR6-9c against neuronal death induced by kainate in rat hippocampus via nuclear and non-nuclear pathways. *J Biol Chem* 2006; **281**: 17432-17445

23 **Pei DS**, Wang XT, Liu Y, Sun YF, Guan QH, Wang W, Yan JZ, Zong YY, Xu TL, Zhang GY. Neuroprotection against ischaemic brain injury by a GluR6-9c peptide containing the TAT protein transduction sequence. *Brain* 2006; **129**: 465-479

24 **Kim KY**, Kim BC, Xu Z, Kim SJ. Mixed lineage kinase 3 (MLK3)-activated p38 MAP kinase mediates transforming growth factor-beta-induced apoptosis in hepatoma cells. *J Biol Chem* 2004; **279**: 29478-29484

25 **Wada T,** Joza N, Cheng HY, Sasaki T, Kozieradzki I, Bachmaier K, Katada T, Schreiber M, Wagner EF, Nishina H, Penninger JM. MKK7 couples stress signalling to G2/M cell-cycle progression and cellular senescence. *Nat Cell Biol* 2004; **6**: 215-226

26 **You HJ,** Pei DS, Sun W. Cloning of human HBx-cDNA and construction of the eukaryotic expression vector. *Acta Academiae Medicinae Xuzhou* 2006; **3**: 191-193

27 **Pan J**, Wang G, Yang HQ, Hong Z, Xiao Q, Ren RJ, Zhou HY, Bai L, Chen SD. K252a prevents nigral dopaminergic cell death induced by 6-hydroxydopamine through inhibition of both mixed-lineage kinase 3/c-Jun NH2-terminal kinase 3 (JNK3) and apoptosis-inducing kinase 1/JNK3 signaling pathways. *Mol Pharmacol* 2007; **72**: 1607-1618

28 **Roux PP**, Dorval G, Boudreau M, Angers-Loustau A, Morris SJ, Makkerh J, Barker PA. K252a and CEP1347 are neuroprotective compounds that inhibit mixed-lineage kinase-3 and induce activation of Akt and ERK. *J Biol Chem* 2002; **277**: 49473-49480

29 **Diao J**, Khine AA, Sarangi F, Hsu E, Iorio C, Tibbles LA, Woodgett JR, Penninger J, Richardson CD. X protein of hepatitis B virus inhibits Fas-mediated apoptosis and is associated with up-regulation of the SAPK/JNK pathway. *J Biol Chem* 2001; **276**: 8328-8340

30 **Kim H**, Lee H, Yun Y. X-gene product of hepatitis B virus induces apoptosis in liver cells. *J Biol Chem* 1998; **273**: 381-385

31 **Wang XW**, Gibson MK, Vermeulen W, Yeh H, Forrester K, Stürzbecher HW, Hoeijmakers JH, Harris CC. Abrogation of p53-induced apoptosis by the hepatitis B virus X gene. *Cancer Res* 1995; **55**: 6012-6016

32 **Jin YM**, Yun C, Park C, Wang HJ, Cho H. Expression of hepatitis B virus X protein is closely correlated with the high periportal inflammatory activity of liver diseases. *J Viral Hepat* 2001; **8**: 322-330

33 **Moriarty AM**, Alexander H, Lerner RA, Thornton GB. Antibodies to peptides detect new hepatitis B antigen: serological correlation with hepatocellular carcinoma. *Science* 1985; **227**: 429-433

34 **Antonucci TK**, Rutter WJ. Hepatitis B virus (HBV) promoters are regulated by the HBV enhancer in a tissue-specific manner. *J Virol* 1989; **63**: 579-583

35 **Hwang GY**, Lin CY, Huang LM, Wang YH, Wang JC, Hsu CT, Yang SS, Wu CC. Detection of the hepatitis B virus X protein (HBx) antigen and anti-HBx antibodies in cases of human hepatocellular carcinoma. *J Clin Microbiol* 2003; **41**: 5598-5603

36 **Wang WL**, London WT, Lega L, Feitelson MA. HBxAg in the liver from carrier patients with chronic hepatitis and cirrhosis. *Hepatology* 1991; **14**: 29-37

37 **Shirakata Y**, Koike K. Hepatitis B virus X protein induces cell death by causing loss of mitochondrial membrane potential. *J Biol Chem* 2003; **278**: 22071-22078

38 **Takada S**, Shirakata Y, Kaneniwa N, Koike K. Association of hepatitis B virus X protein with mitochondria causes mitochondrial aggregation at the nuclear periphery, leading to cell death. *Oncogene* 1999; **18**: 6965-6973

39 **Zhang SJ**, Chen HY, Chen ZX, Wang XZ. Possible mechanism for hepatitis B virus X gene to induce apoptosis of hepatocytes. *World J Gastroenterol* 2005; **11**: 4351-4356

40 **Lin N**, Chen HY, Li D, Zhang SJ, Cheng ZX, Wang XZ. Apoptosis and its pathway in X gene-transfected HepG2 cells. *World J Gastroenterol* 2005; **11**: 4326-4331

41 **Lok J**, Martin LJ. Rapid subcellular redistribution of Bax precedes caspase-3 and endonuclease activation during excitotoxic neuronal apoptosis in rat brain. *J Neurotrauma* 2002; **19**: 815-828

42 **Perfettini JL**, Reed JC, Israël N, Martinou JC, Dautry-Varsat A, Ojcius DM. Role of Bcl-2 family members in caspase-independent apoptosis during Chlamydia infection. *Infect Immun* 2002; **70**: 55-61

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BRIEF ARTICLE

Magnetic resonance imaging: A new tool for diagnosis of acute ischemic colitis?

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Abstract

AIM: To define the evolution of ischemic lesions with 7T magnetic resonance imaging (7T-MRI) in an animal model of acute colonic ischemia.

METHODS: Adult Sprague-Dawley rats were divided into two groups. Group Ⅰ underwent inferior mesenteric artery (IMA) ligation followed by macroscopic observations and histological analysis. In group Ⅱ, 7T-MRI was performed before and after IMA ligation and followed by histological analysis.

RESULTS: Morphological alterations started to develop 1 h after IMA ligation, when pale areas became evident in the splenic flexure mesentery and progressively worsened up to 8 h thereafter, when the mesentery was less pale, and the splenic flexure loop appeared very dark. The 7T-MRI results reflected these alterations, showing a hyperintense signal in both the intraperitoneal space and the colonic loop wall 1 h after IMA ligation; the latter progressively increased to demonstrate a reduction in the colonic loop lumen at 6 h. Eight hours after IMA ligation, MRI showed a persistent colonic mural hyperintensity associated with a reduction in peritoneal free fluid. The 7T-MRI findings were correlated with histological alterations, varying from an attenuated epithelium with glandular apex lesions at 1 h to coagulative necrosis and loss of the surface epithelium detected 8 h after IMA ligation.

CONCLUSION: MRI may be used as a substitute for invasive procedures in diagnosing and grading acute ischemic colitis, allowing for the early identification of pathological findings.

Key words: Ischemic colitis; Animal models; Sprague-Dawley rats; Magnetic resonance imaging; Histopathology

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INTRODUCTION

Ischemic colitis (IC) was first described by Boley *et al*[1]

and Theodoropoulou *et al*[2], and is a relatively common disease, being the most frequent form of intestinal ischemia[3] and the second-most frequent cause of lower gastrointestinal bleeding[4]. IC is the consequence of an acute interruption or chronic decrease in the colonic blood supply[5], which may be either occlusive or non-occlusive in origin[2]. This disease results in ischemic necrosis of variable severity that can range from superficial mucosal involvement to full-thickness transmural necrosis[5,6].

Clinically, IC presents in either a gangrenous (acute fulminant) or non-gangrenous form (acute transient; chronic)[2], with a mortality rate ranging from 10% for the non-occlusive disease to 90% for occlusive mesenteric infarction due to embolus or thrombosis[5]. The left colon is involved in 75% of cases and the right colon in the remaining 25%; the splenic flexure and the sigmoid colon are the areas most frequently affected[3,7].

The presentation of IC is not specific and is highly variable, therefore, its diagnosis largely depends on clinical suspicion[7]. In this context, the role of imaging techniques is controversial. Standard radiology and computed tomography (CT), the latter considered the best diagnostic modality in acute settings[8,9], yield non-specific and late findings, whereas the advantages of magnetic resonance imaging (MRI) are still a matter of debate[5].

The functional and morphological responses to ischemia produced by inferior mesenteric artery (IMA) ligation have been studied in the canine, porcine and rat colon[10-13]. However, to date, nothing is known about the histological evolution of the colonic ischemic injury or the relationship between these lesions and MRI findings. Therefore, the aim of our study was to define the evolution of histological ischemic lesions and to compare anatomopathological features with T2-weighted MRI in an animal model of colonic ischemia.

MATERIALS AND METHODS

Animal preparation

All procedures performed on animals were approved by our Institutional Animal Care and Use Committee. Nine adult male Sprague-Dawley rats (250-340 g; Harlan Laboratories, Indianapolis, IN, United States) were used in this study. The rats were maintained on a 12/12 h light/dark cycle and allowed free access to food and water. They were anesthetized with ketamine (100 mg/kg i.m.) and medetomidine (0.25 mg/kg i.m.) injections. Butorphanol (0.1 mg/kg s.c.) was used immediately before the intervention to ensure intraoperative analgesia. Further injections of these drugs were provided throughout the intervention to maintain a sufficient state of anesthesia. Each rat was allowed to breathe spontaneously. Body temperature was monitored with a rectal probe and maintained at 37.0 ± 0.5 ℃ with a heating blanket regulated by a homeothermic blanket control unit (Harvard Apparatus Ltd., Holliston, MA, United States).

After drug injection, eight rats were prepared for surgery through thoracic and abdominal trichotomy. These areas were then washed with povidone iodine and alcohol. These animals were divided at random into two groups of four rats each. Group I rats underwent IMA ligation followed by macroscopic observation and histological analysis. Before and after IMA ligation, group II rats underwent 7-tesla (7T) MRI followed by histological analysis of a colon specimen after euthanasia. A healthy rat underwent an MRI bowel enema without IMA ligation to enable us to study its bowel anatomy.

Surgical procedures

After a midline laparotomy, the small and large bowel were exposed from the abdominal cavity and displaced to the left. A photograph of the physiological appearance of the bowel and mesentery was taken with a digital camera (Panasonic Lumix DMC-TZ8, 12.0 megapixels, ISO 2000; Osaka, Japan). The IMA was identified and ligated at the origin with Prolene 7/0 and sectioned.

Macroscopic analysis

After IMA ligation, the anesthetized rats of group I were observed for 8 h. The bowel was exposed on gauze moistened with saline to prevent excessive evaporative loss. Photographs of the exposed bowel and mesentery were taken every 10 min for the first hour and every 30 min thereafter. At the end of the observation period, all rats were euthanized with an intrapulmonary injection of Tanax (0.5 mL), and the large bowel was excised for histological analysis.

MRI

Abdominal 7T-MRI scans were acquired in group II animals (Bruker BioSpec 70/16US; Bruker Medical Systems, Ettlingen, Germany) before IMA ligation to record the physiological appearance of the abdomen. After IMA ligation, the entire intestine was replaced in the abdomen, and the abdominal wall was closed with Vicryl 2/0 thread.

Each rat underwent 7T-MRI at 1 h, 4 h, 6 h and 8 h after IMA ligation as follows: Tripilot sequence, parameters: TR 100.0 ms; TE 6.0 ms; FOV 8.00 cm; IS 2.00 mm; N slice 3 and RareT2 sequence in axial section, parameters: TR 6060.3 ms; TE 36.0 ms; FOV 7.00 cm; matrix 256 × 256; IS 1.00/1.00 mm; N slice 52; acquisition time: 14 min, 32 s, 688 ms. To obtain bowels for histological analysis, selected rats were sacrified at 1 h, 4 h, 6 h and 8 h after 7T-MRI.

Histological analysis

For light microscopy, the large bowels in both groups of rats were excised from the cecum to the rectum, including the mesentery, and stored in 10% buffered formalin acetate for at least 2 d. The samples were divided into three segments: the cecum and proximal colon (first segment), the splenic flexure (second segment), and the distal colon and rectum (third segment). Sections 3 mm in size were obtained at 10-mm intervals from each segment and embedded in paraffin. Transverse sections 3 μm thick were cut and stained with hematoxylin-eosin. The sections were mounted on chrome alum/gelatin-coated slides,

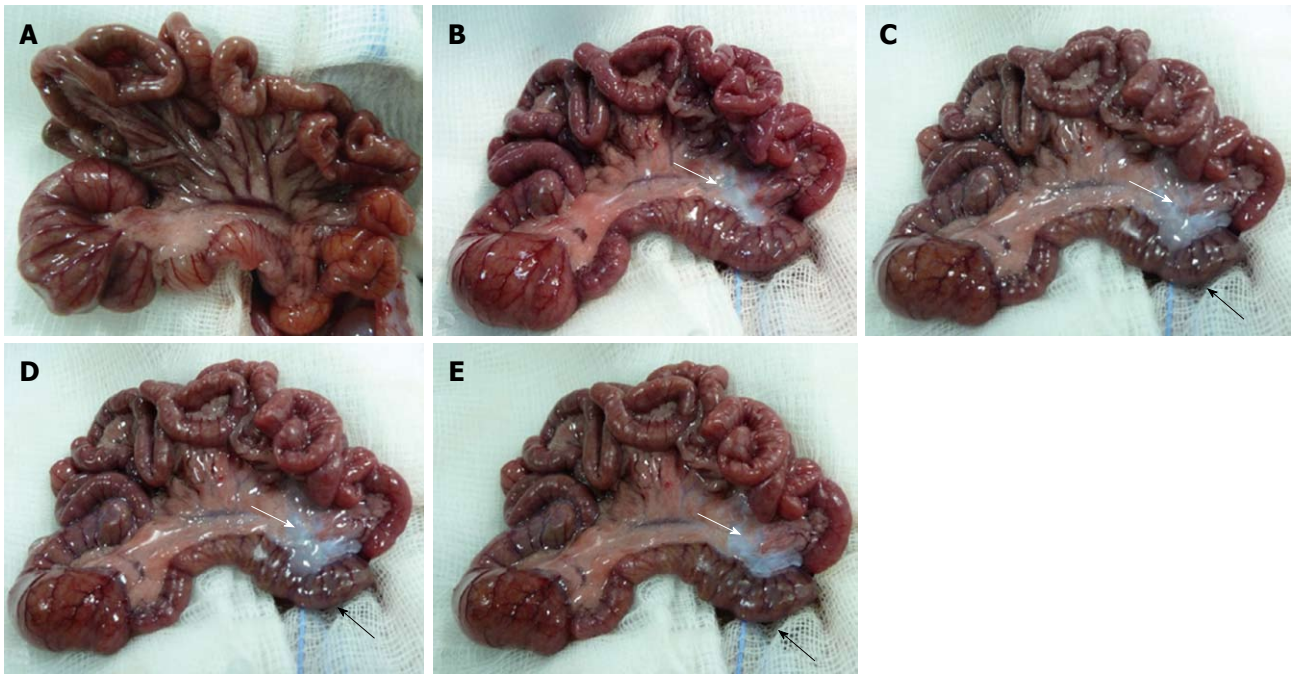

Figure 1 Macroscopic monitoring. A: Physiological appearance of the rat bowel; B: Rat bowel 1 h after inferior mesenteric artery (IMA) ligation; C: At 4 h after IMA ligation; D: At 6 h after IMA ligation; E: At 8 h after IMA ligation.

dehydrated and coverslipped. Slides were imaged with a Zeiss Axio Skope microscope equipped with a high-resolution digital camera (ORCA-HR C4742-95-12HR, 10 MP; Hamamatsu Photonics, Hamamatsu, Japan)

MRI bowel enema

This procedure was performed on one healthy rat to determine the large bowel anatomy and topography. Under anesthesia, a 16 G angiocatheter was inserted in the rectum and fixed to the anal skin by a purse-string suture. After the injection of 8-10 mL warm water, 7T-MRI was performed using the following protocol: Tripilot sequence, parameters: TR 100.0 ms; TE 6.0 ms; FOV 8.00 cm; IS 2.00 mm; N slice 3 and RareT2 sequence in axial section, parameters: TR 6060.3 ms; TE 36.0 ms; FOV 6.00 cm; matrix 256 × 256; IS 1.00/1.00 mm; N slice 44; acquisition time: 14 min, 32 s, 688 ms. These scans served as anatomic images of reference for comparison purposes to identify ischemic damage in group Ⅱ rats.

RESULTS

Macroscopic evaluation

Figure 1A shows the appearance of a rat intestine immediately before IMA ligation; the colon and the ileum were of normal size and presented a uniform serous membrane and rose-colored mesentery. One hour after IMA ligation, pale areas appeared in the splenic flexure mesentery (indicated by a white arrow in Figure 1B); these areas progressively increased (white arrow) and were associated, four hours after IMA ligation, with a change in the color of the splenic flexure loop (black arrow), which appeared dark reddish blue (Figure 1C). Six hours after IMA ligation, the splenic flexure mesentery was very pale (white arrow), and the loop was very dark (black arrow, Figure 1D). Eight hours after IMA ligation, although the loop was even darker (black arrow), the mesentery was less pale (white arrow, Figure 1E). No other macroscopic alterations were noted during the 8 h observation period, and the chronological sequence of the macroscopic morphological changes was the same in all group Ⅰ animals.

MRI

Figure 2A shows a 7T-MRI abdominal scan of a group Ⅱ rat immediately before IMA ligation. No pathological findings related to ischemic damage were detected. One hour after IMA ligation, the T2 sequences showed minimal findings, namely, hyperintense signals in both the colonic loop wall (arrow) and the intraperitoneal space (curved arrow, Figure 2B). These MRI findings were more pronounced four hours after IMA ligation, suggesting colonic wall edematous thickening and a small amount of peritoneal free fluid (Figure 2C). Six hours after ligation, the amount of peritoneal free fluid increased (curved arrow), as did the colonic wall hyperintensity (arrow, Figure 2D). Eight hours after IMA ligation, MRI showed persistent colonic mural hyperintensity (arrow) associated with a reduction in the peritoneal free fluid (curved arrow), probably related to compensatory intraperitoneal drainage (Figure 2E).

MRI colon enema

Figure 2F shows the hyperintense signals of the splenic flexure (arrow), descending colon (curved arrow) and rectum (star) distended by instilled water. Comparison

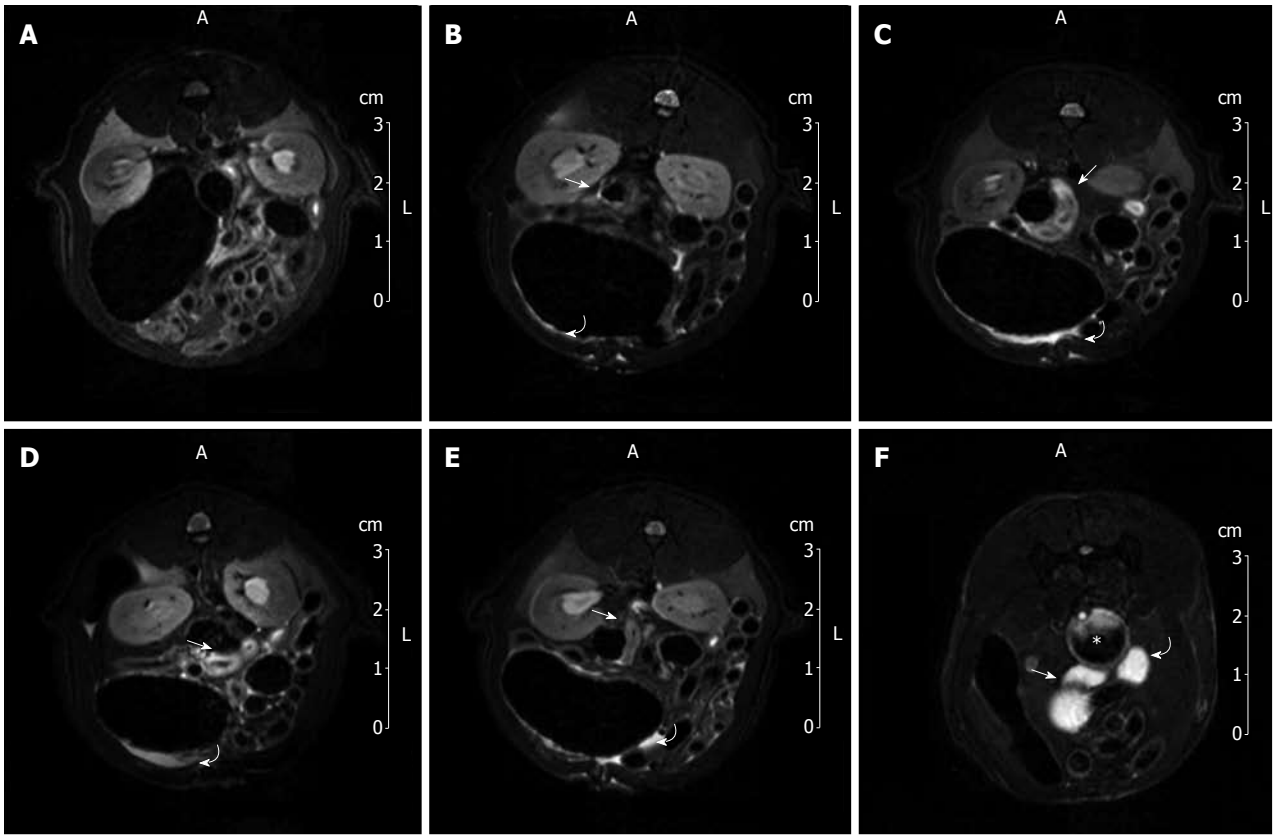

Figure 2 7T magnetic resonance imaging investigation. A: Image of a 7T magnetic resonance imaging (MRI) abdominal scan before inferior mesenteric artery (IMA) ligation; B: A 7T MRI abdominal scan 1 h after IMA ligation; C: At 4 h after IMA ligation; D: At 6 h after IMA ligation; E: At 8 h after IMA ligation; F: Image of 7T MRI colon enema.

of the 7T-MRI and MRI colon enema scans reveals that the splenic flexure was the colonic area most affected by ischemia.

Histological analysis

Histology revealed anatomopathological alterations in the second segments (colonic splenic flexure), whereas the first and third segments were unaffected. The histology of the colonic sections obtained one hour after IMA ligation from the proximal colon and the splenic flexure are shown in Figure 3A and B, respectively. In contrast to the normal histological pattern of the proximal colon, the splenic flexure section showed an attenuated epithelium with glandular apex lesions (star). Four hours after IMA ligation, large ischemic lesions appeared at the splenic flexure level: superficial epithelium nuclear pyknosis and epithelium mucin depleted with submucosal edema (star) leading to vessel collapse (arrow, Figure 3C). Six hours after ligation, in the same colonic area, we found a further reduction in crypt goblet cells, clear signs of apoptosis such as nuclear pyknosis and hypereosinophilia, and epithelial regeneration attempts, as shown by the presence of mitotic figures (arrow, Figure 3D). At the end of the observation (8 h), ischemic injury of the splenic flexure was more pronounced; necrosis and a loss of the surface epithelium, crypt drop-out (arrow) with a depletion of goblet cells (Figure 3E) and a markedly edematous submucosa (star, Figure 3F) were observed. Signs of regeneration in response to the injury appeared at the bottom of the crypts (arrow, Figure 3G) near the images of coagulative necrosis (Figure 3H). Ischemic changes were related to vascular anatomy, and a sharp demarcation line often separated the involved from the uninvolved mucosa.

DISCUSSION

The availability of animal models in which to reproduce human diseases has increased our knowledge of human physiopathology and led to new diagnostic and therapeutic approaches[14]. This study, based on an animal model of colonic ischemic damage, was designed to define the evolution of histological ischemic lesions and to compare anatomopathological features with corresponding 7T-MRI findings. Thus far, the chronological sequence of early colonic ischemic damage has not been described, and the role of MRI is still widely debated. To the best of our knowledge, this is the first study using 7T-MRI for the instrumental evaluation of a human disease reproduced in a rat animal model. We decided to use T2-weighted MRI sequences because, as shown in a previous study[15], they allow us to identify, without using contrast media, the hyperintensity of parietal edema, which is an early sign of intestinal ischemia.

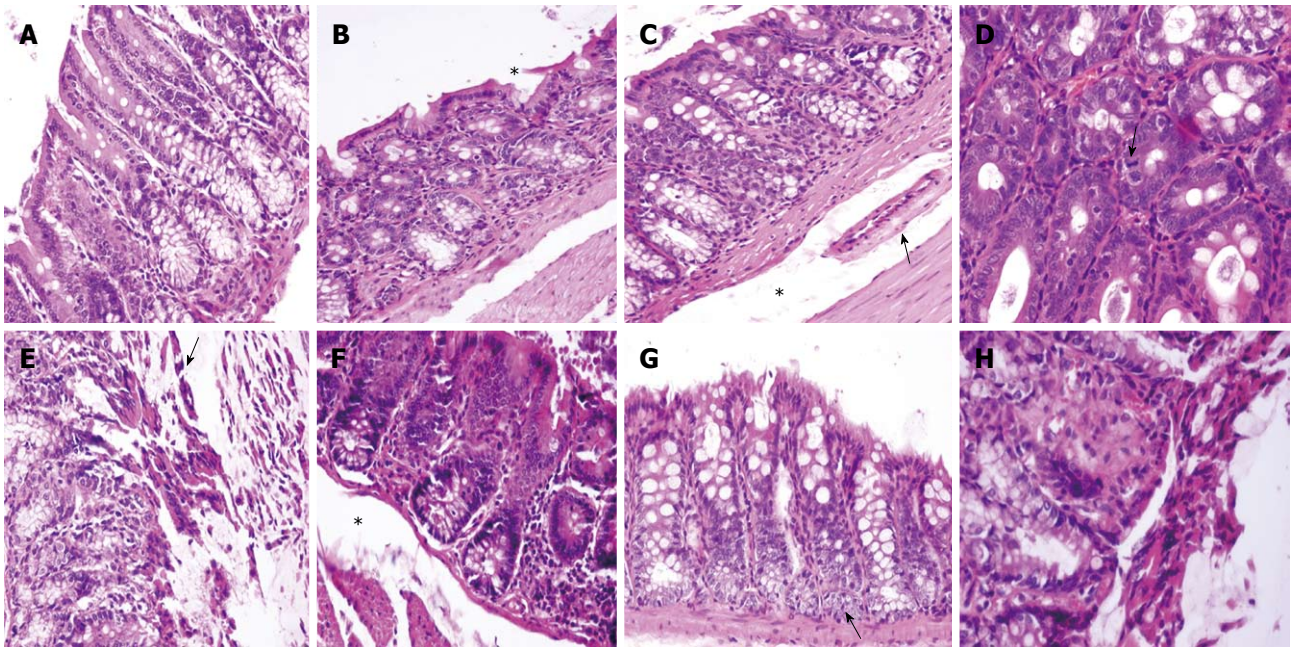

Figure 3 Histological analysis. A: Normal histological pattern of the rat proximal colon; B: Histological pattern of the rat proximal colon 1 h after inferior mesenteric artery (IMA) ligation; C: At 4 h after IMA ligation; D: At 6 h after IMA ligation; E: At 8 h after IMA ligation; note the necrosis and loss of the surface epithelium; F: Markedly edematous submucosa (star); G: Active regeneration signs at the bottom of the crypts; H: Coagulative necrosis.

We identified anatomopathological alterations as early as one hour after IMA ligation, namely, a pale mesentery and mild surface epithelium damage at the colonic splenic flexure level. Additionally at one hour after ligation, the MRI study showed a small amount of peritoneal free fluid associated with mild edematous thickening of the colonic splenic flexure wall. Moreover, there was a relevant correlation between the MRI changes observed during the course of bowel ischemia and the pathological damage. Indeed, the morphological and MRI findings showed a progressive and parallel worsening with time up to eight hours when, despite the change in the color of the colonic loop, we observed a mild improvement in mesenteric color associated with a reduction in peritoneal free fluid. Therefore, MRI detected very early signs of colonic ischemia and allowed us to monitor the evolution of the ischemic damage. Consequently, although CT remains a valid diagnostic tool for the visualization of early signs of bowel ischemia and evaluation of colonic ischemic damage[8,9,16], MRI, which does not require the use of a contrast medium or ionizing radiation, can play an important role in the diagnostic and therapeutic management of patients with acute colitis ischemia.

We performed MRI colon enema in a surgically untreated rat to define the large bowel anatomy and topography. We were thus able to identify the specific colonic hyperintensity area detected by MRI after IMA ligation as the colonic splenic flexure. In agreement with a previous study, although the superior mesenteric artery is more important than the IMA in maintaining the cecum and transverse colon perfusion, ligation of the IMA alone produced significant injury in a single, specific colonic area[10]. The major vulnerability of the splenic flexure to ischemic damage may be related to the presence of more limited collateral networks in this region, apparently representing a "watershed" area where the two circulations meet[2]. In this context, the amount of ischemic injury depends on the high variability of the vascular anatomy[7].

The rat model used in this study had several limitations: vascular occlusion was sudden and total, whereas partial occlusion is possible in humans; IMA ligation was performed at the emergence of the vessel, whereas in clinical practice, distal occlusions are also observed; the time window analysis was limited to 8 h, but these patients often reach the hospital a few days after the original occlusive event. However, despite the limitations of the animal model, this study shows that MRI is useful in the diagnosis of acute IC. Moreover, although our MRI scans were performed with a 7T-MRI machine, which is not yet available in clinical practice, both our results and those of previous studies suggest that 7T MRI machines are appropriate for clinical research on humans[17,18].

In conclusion, the assumption of parallels between the experimental colonic ischemic damage in this animal model and humans is reasonable. Our results indicate that MRI allows for the identification of pathological findings of acute IC and their correlation with histopathological features. Therefore, MRI can play a relevant role in the diagnostic management of acute IC and may be substituted for other invasive surgical and endoscopic procedures in diagnosing and grading IC when ischemic injury is suggested. The possibility of assessing the evolution of IC over time and correlating the histological alterations with imaging patterns could result in a more accurate and earlier identification of imaging diagnostic features and thus facilitate more effective treatment.

COMMENTS

Background

Ischemic colitis (IC) is a relatively common disease, being the most frequent form of intestinal ischemia and the second-most frequent cause of lower gastrointestinal bleeding. Presentation of IC is not specific and is highly variable, therefore, the diagnosis largely depends on clinical suspicion. In this context, the role of imaging techniques remains controversial.

Research frontiers

The role of magnetic resonance imaging (MRI) in the diagnostic management of acute IC is still a matter of debate, and nothing is known about the histological evolution of the acute colonic ischemic injury or the relationship between these lesions and MRI findings.

Innovations and breakthroughs

To be known, this is the first study using 7T-MRI for the instrumental evaluation of a human disease reproduced in a rat animal model. In this study, authors established the chronological evolution of the early functional and morphological responses to ischemia produced by inferior mesenteric artery (IMA) ligation and compared their anatomopathological features with the corresponding 7T-MRI findings.

Applications

The possibility of detecting the early signs of colonic ischemic injury with MRI suggests that this technique can play an important role in diagnosing and grading acute IC.

Terminology

7T-MRI: A non-invasive method of demonstrating internal anatomy based on the principle that atomic nuclei in a strong magnetic field (7 Tesla) absorb pulses of radiofrequency energy and emit them as radio waves that can be reconstructed into computerized images. The concept includes proton-spin tomographic techniques; IC: Inflammation of the colon due to colonic ischemia resulting from alterations in systemic circulation or local vasculature.

Peer review

This is a good demonstration of the potential use of T2-weighted MRI for detecting edema in IC. In an experimental model, results of MRI are compared with physiological appearance and histology of the bowel at various times after IMA ligation.

REFERENCES

1 **Boley SJ**, Schwartz S, Lash J, Sternhill V. Reversible vascular occlusion of the colon. *Surg Gynecol Obstet* 1963; **116**: 53-60

2 **Theodoropoulou A**, Koutroubakis IE. Ischemic colitis: clinical practice in diagnosis and treatment. *World J Gastroenterol* 2008; **14**: 7302-7308

3 **Paterno F**, Longo WE. The etiology and pathogenesis of vascular disorders of the intestine. *Radiol Clin North Am* 2008; **46**: 877-885

4 **Taourel P**, Aufort S, Merigeaud S, Doyon FC, Hoquet MD, Delabrousse E. Imaging of ischemic colitis. *Radiol Clin North Am* 2008; **46**: 909-924

5 **Stamatakos M**, Douzinas E, Stefanaki C, Petropoulou C, Arampatzi H, Safioleas C, Giannopoulos G, Chatziconstantinou C, Xiromeritis C, Safioleas M. Ischemic colitis: surging waves of update. *Tohoku J Exp Med* 2009; **218**: 83-92

6 **Baixauli J**, Kiran RP, Delaney CP. Investigation and management of ischemic colitis. *Cleve Clin J Med* 2003; **70**: 920-921

7 **Elder K**, Lashner BA, Al Solaiman F. Clinical approach to colonic ischemia. *Cleve Clin J Med* 2009; **76**: 401-409

8 **Angelelli G**, Scardapane A, Memeo M, Stabile Ianora AA, Rotondo A. Acute bowel ischemia: CT findings. *Eur J Radiol* 2004; **50**: 37-47

9 **Romano S**, Romano L, Grassi R. Multidetector row computed tomography findings from ischemia to infarction of the large bowel. *Eur J Radiol* 2007; **61**: 433-441

10 **Leung FW**, Su KC, Pique JM, Thiefin G, Passaro E, Guth PH. Superior mesenteric artery is more important than inferior mesenteric artery in maintaining colonic mucosal perfusion and integrity in rats. *Dig Dis Sci* 1992; **37**: 1329-1335

11 **Beuk RJ**, Heineman E, Tangelder GJ, Kurvers HA, Bonke HJ, oude Egbrink MG. Effects of different durations of total warm ischemia of the gut on rat mesenteric microcirculation. *J Surg Res* 1997; **73**: 14-23

12 **Matthews JG**, Parks TG. Ischaemic colitis in the experimental animal. I. Comparison of the effects of acute and subacute vascular occlusion. *Gut* 1976; **17**: 671-676

13 **Acosta S**, Nilsson TK, Bergqvist D, Björck M. Activation of fibrinolysis and coagulation in non-occlusive intestinal ischaemia in a pig model. *Blood Coagul Fibrinolysis* 2004; **15**: 69-76

14 **Grassi R**, Cavaliere C, Cozzolino S, Mansi L, Cirillo S, Tedeschi G, Franchi R, Russo P, Cornacchia S, Rotondo A. Small animal imaging facility: new perspectives for the radiologist. *Radiol Med* 2009; **114**: 152-167

15 **Kim MY**, Suh CH, Kim ST, Lee JH, Kong K, Lim TH, Suh JS. Magnetic resonance imaging of bowel ischemia induced by ligation of superior mesenteric artery and vein in a cat model. *J Comput Assist Tomogr* 2004; **28**: 187-192

16 **Balthazar EJ**, Yen BC, Gordon RB. Ischemic colitis: CT evaluation of 54 cases. *Radiology* 1999; **211**: 381-388

17 **Kraff O**, Theysohn JM, Maderwald S, Kokulinsky PC, Dogan Z, Kerem A, Kruszona S, Ladd ME, Gizewski ER, Ladd SC. High-resolution MRI of the human parotid gland and duct at 7 Tesla. *Invest Radiol* 2009; **44**: 518-524

18 **Kraff O**, Bitz AK, Kruszona S, Orzada S, Schaefer LC, Theysohn JM, Maderwald S, Ladd ME, Quick HH. An eight-channel phased array RF coil for spine MR imaging at 7 T. *Invest Radiol* 2009; **44**: 734-740

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BRIEF ARTICLE

Diagnostic yield of small bowel capsule endoscopy depends on the small bowel transit time

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Abstract

AIM: To investigate whether the small bowel transit time (SBTT) influences the diagnostic yield of capsule endoscopy (CE).

METHODS: Six hundred and ninety-one consecutive CE procedures collected in a database were analyzed. SBTT and CE findings were recorded. A running mean for the SBTT was calculated and correlated to the diagnostic yield with a Spearman's correlation test. Subgroup analyses were performed for the various indications for the procedure.

RESULTS: There was a positive correlation between the diagnostic yield and SBTT (Spearman's rho 0.58, $P < 0.01$). Positive correlations between diagnostic yield and SBTT were found for the indication obscure gastrointestinal bleeding ($r = 0.54$, $P < 0.01$), for polyposis and carcinoid combined ($r = 0.56$, $P < 0.01$) and for the other indications (r = 0.90, $P < 0.01$), but not for suspected Crohn's disease ($r = -0.40$).

CONCLUSION: The diagnostic yield in small bowel capsule endoscopy is positively correlated with the small bowel transit time. This is true for all indications except for suspected Crohn's disease.

Key words: Capsule endoscopy; Small bowel transit time; Diagnostic yield

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INTRODUCTION

Capsule endoscopy (CE) is a very sensitive diagnostic technique to detect small bowel pathology. It has a higher diagnostic yield than conventional diagnostic methods, i.e., push enteroscopy, small-bowel follow-through, conventional CT and angiography[1]. The reported diagnostic yield of CE varies between 38% and 83%[2-11]. In 15%-20% of all CE's the capsule does not reach the cecum within recording time. Risk factors for incomplete CE procedures include previous small-bowel surgery, hospitalization, moderate or poor bowel cleansing, and a gastric transit time longer than 45 min[12].

For a good and complete evaluation of the small

bowel, the capsule should reach the cecum within recording time, which is eight to eleven hours depending on the manufacturer. Therefore, some investigators use a prokinetic agent to speed up the gastric and small bowel transit. However, the short bowel transit time (SBTT) may influence the diagnostic yield of CE. With colonoscopy, the detection rate of neoplastic lesions is higher when the time to withdraw the colonoscope is longer[13-15]. It is conceivable that a similar principle also applies for small bowel CE. We therefore hypothesize that the diagnostic yield of CE depends on the small bowel transit time. To study this, we analyzed the influence of small bowel transit time on the diagnostic yield of CE in 691 consecutive procedures performed in our department.

MATERIALS AND METHODS

Data from all consecutive CE studies performed at the University Medical Center Groningen, the Netherlands, between September 2003 and January 2009 were collected. Data that were collected included patient demographics, indications for the procedure, procedural data, including gastric transit time (GTT) and SBTT, and findings of the procedure. The GTT was defined as the time, in minutes, from the first image of the stomach until the first image of the duodenum. The SBTT was defined as the passage time, in minutes, from the first image of the duodenum until the first image of the cecum. If the capsule did not reach the cecum within recording time, the SBTT was recorded as the time during which small bowel images were captured. CE was considered complete when the cecum was reached within recording time.

CE procedure

During the study period, all patients received bowel preparation. Patients were given standardized instructions before the procedure, and informed consent was obtained. The patients were asked to stop iron supplements seven days before CE and to use a low-fiber diet three days before CE. The patients started a fasting period at midnight before the procedure. Bowel preparation consisted of the ingestion of four liters of a polyethylene glycol solution (Colofort®), 3 L the evening before the procedure and 1 L in the morning. The capsule (Pillcam; Given Imaging Ltd, Yoqneam, Israel) was swallowed in the morning. The patients were allowed to drink fluids after three hours and to consume a light meal after five hours. Before capsule ingestion, 10 mL of antifoam and a prokinetic agent was given, 10 mg of domperidone (before July 1st 2008, $n = 641$) or 250 mg of erythromycin (after July 1st 2008, $n = 50$). All CE recordings were reviewed by two gastroenterologists, experienced with CE (RKW and JJK). Controversial findings were discussed, and consensus was reached upon the final diagnosis. The most relevant findings obtained from CE were documented and categorized according to standard terminology[16] as angiectasia(s); ulcer(s); bleeding of unknown origin; erosion (s); polyp(s)/tumor(s); incidental abnormality of esophagus, stomach, or colon; no abnormality; or unable to make a diagnosis.

Statistical analysis

The SBTT was not normally distributed (tested with a Kolmogorov-Smirnov test) in the study population. To demonstrate the correlation between average diagnostic yield and average SBTT, the average yield was calculated of 50 consecutive transit times and plotted. Diagnostic yield was expressed as 0 for absence of abnormalities and 1 for presence of abnormalities. In this way, a running mean for the SBTT was calculated for 50 consecutive patients and correlated to the diagnostic yield with a Spearman's correlation test. A rho's correlation coefficient was calculated. Comparison of SBTT between groups was performed using a Mann-Whitney *U* test.

Subgroup analyses were performed for the various indications for the procedure. *P*-values below 0.05 were considered significant. SPSS 14.0 for Windows software (SPSS Inc., Chicago, IL, United States) and Microsoft Office Excel 2003 were used for statistical analyses.

RESULTS

Six hundred and ninety-one consecutive CE procedures were analyzed. The mean age of the patients was 54 years (range 9-93, SD 18 years). 55% of the patients were male. Indications for CE were obscure gastrointestinal bleeding (OGIB) (67%), suspected Crohn's disease (22%), polyposis (4%), carcinoid (3%) and other (4%). CE findings were as follows in the investigated patients: angiectasia(s) in 121 cases (18%), ulcer(s) in 42 (6%), erosion(s) in 83 (12%), bleeding of unknown origin in 30 (4%), polyp(s)/tumor(s) in 56 (8%), abnormality of esophagus, stomach, or colon in 15 (2%), stenosis in 2 (0.3 %), unable to make a diagnosis in 6 (1%) and no abnormalities in 336 cases (48%). Overall, the diagnostic yield was 51%.

The cecum was reached in 82% of all procedures. The overall median small bowel transit time was 246 min (25 and 75 percentiles: 190 and 342). In CE cases with positive findings, the median SBTT was 254 min (25 and 75 percentiles: 200 and 361), in negative CE procedures, the median SBTT was 239 (25 and 75 percentiles 178 and 320), this difference was significant ($P = 0.012$). There was a positive correlation between the diagnostic yield and SBTT (Figure 1) indicating that the longer the SBTT, the higher the diagnostic yield (Spearman's rho 0.58, $P < 0.01$).

Next, patients were excluded in whom the cecum was not reached ($n = 125$) within recording time, leaving 566 procedures with complete visualization of the small intestine. The overall median SBTT was 233 min (25 and 75 percentiles: 178 and 295). In cases with positive findings, the median SBTT was 236 (25 and 75 percentiles: 186 and 300), in negative CE procedures the median SBTT was 229 min (25 and 75 percentiles: 121 and 281), this difference was not significant ($P = 0.078$). A positive correlation was again observed between the diagnostic yield and SBTT (Spearman's rho 0.40, $P < 0.01$, Figure 2).

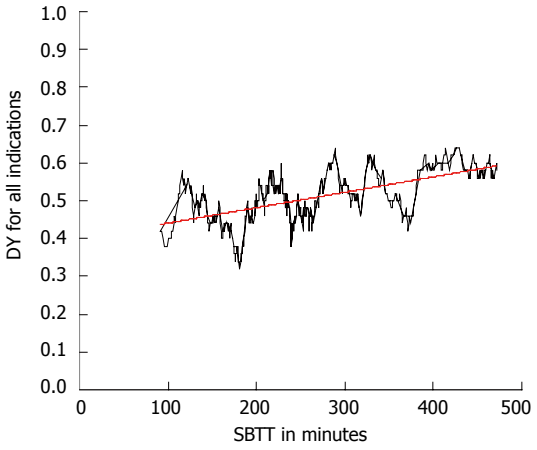

Figure 1 Correlation between the small bowel transit time in minutes and the diagnostic yield for all patients. Spearman's rho coefficient 0.58 ($P <$ 0.01) shown by the black line. The trend of this correlation is shown by the red line. DY: Diagnostic yield; SBTT: Small bowel transit time.

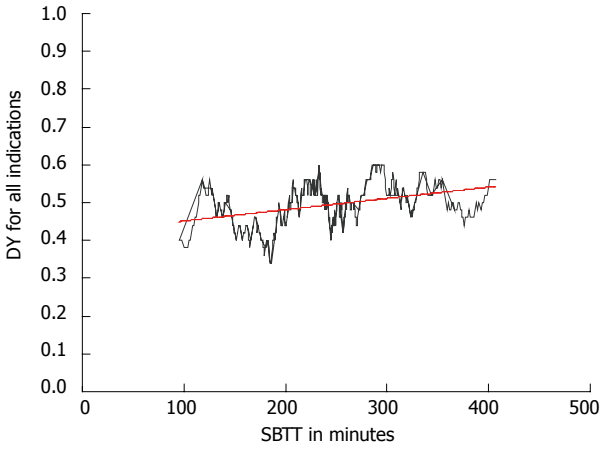

Figure 2 Correlation between the small bowel transit time in minutes and the diagnostic yield in patient with complete capsule endoscopy. Spearman's rho coefficient 0.40 ($P < 0.01$) shown by the black line. The trend of this correlation is shown by the red line. DY: Diagnostic yield; SBTT: Small bowel transit time.

Subgroup analysis for the different indications was performed for OGIB, suspected Crohn's disease, polyposis and carcinoid combined and other indications. The indications polyposis and carcinoid were taken together because both groups were too small for separate subgroup analysis. For these indications, positive correlations between diagnostic yield and SBTT were found for OGIB ($r = 0.54$, $P < 0.01$), for polyposis plus carcinoid ($r = 0.56$, $P < 0.01$) and for the other indications ($r = 0.90$, $P < 0.01$). However this was not observed for Crohn's disease ($r = -0.40$). These results are depicted in Figure 3.

DISCUSSION

In this study, we found a positive correlation between the diagnostic yield of CE and small bowel transit time, irrespective of whether the capsule had reached the cecum within recording time. These findings are in accordance with those from colonoscopy studies, which show higher diagnostic yields for detecting neoplastic lesions when the withdrawal time during colonoscopy is longer[10-13] and from one previous study on the effect of SBTT on the diagnostic yield of CE[17]. Most of these colonoscopy studies divided the withdrawal time into more or less than a chosen number of minutes. In CE there are no known standard SBTT times, so we judged that it would not be correct to randomly divide the SBTT in two or more randomly chosen groups. Therefore we calculated a running mean to determine whether the diagnostic yield correlated with the SBTT. We found a positive correlation between the two, meaning that a longer transit time, implicating more images of the small bowel, resulted in a higher diagnostic yield.

For colonoscopy, the correlation between diagnostic yield and withdrawal time was only investigated in subjects undergoing screening for neoplastic lesions. In this study we looked at all indications for CE. We found a positive correlation between the diagnostic yield and SBTT for the indications OGIB and polyposis/carcinoid and for other indications, but not for the indication suspected Crohn's disease. The latter is probably due to the multiple and widespread small bowel lesions usually seen in Crohn's disease. Therefore the endoscopist may be less dependent upon the mucosal inspection time to make the diagnosis. Furthermore, a previous study showed reduced capsule transit times in Crohn's disease[18].

What does this positive correlation between diagnostic yield and SBTT mean for clinical practice? CE is less valuable when the cecum is not reached within recording time, but on the other hand our study indicates that the diagnostic yield is lower when the SBTT is shorter. So, ideally, the SBTT should be as long as possible, yet the capsule should reach the cecum within recording time. The development of capsule systems with longer battery times may be helpful. One important issue in this matter is whether there is a role for prokinetic agents in CE. In this way, one could influence the small bowel transit time. There is no consensus on this subject[1]. In most of the available studies, there are no data on the influence of prokinetics on the diagnostic yield of CE. Taking our data into account, it may not be wise to use prokinetics that speed up the SBTT. However this must be weighed against the fact that a prolonged GTT is a risk factor for incomplete CE[12]. It may therefore be useful to use an agent which shortens GTT without influencing SBTT.

A well known prokinetic agent is erythromycin. It induces high amplitude gastric propulsive contractions by activating gastric interdigestive migrating motor complexes, thereby accelerating gastric emptying[19-22]. The effects of erythromycin on SBTT are unclear. In the most recent publication on this subject, erythromycin reduced the GTT but had no significant effect on SBTT, total bowel transit time and CE completing rates[22]. Previous studies found similar results[23,24], but in one at the cost of visibility[24]. Others found no effect of erythromycin on either GTT or SBTT[25]. Overall, the data are not very ro-

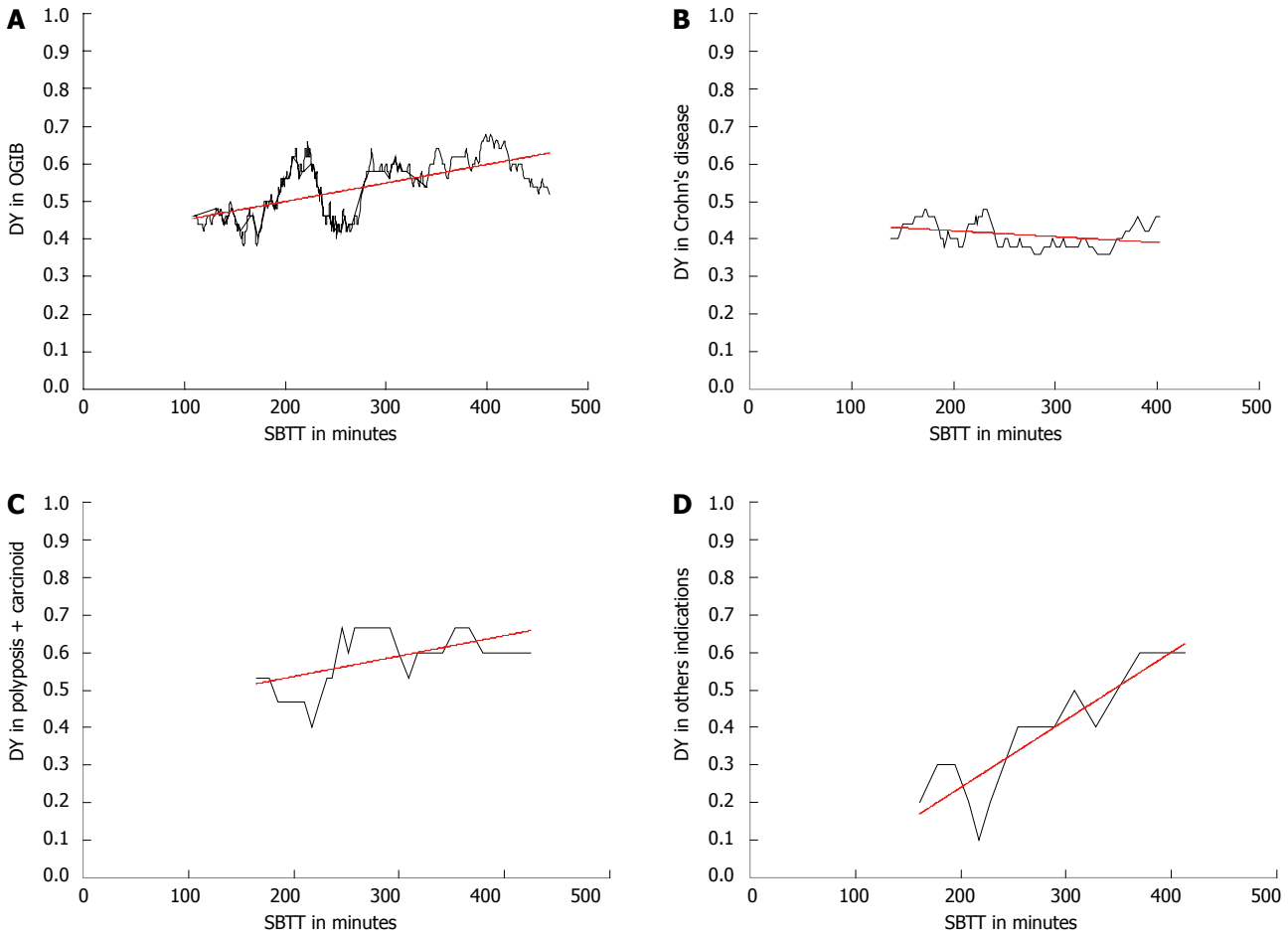

Figure 3 Correlations between small bowel transit time and diagnostic yield. A: Obscure gastrointestinal bleeding (OGIB; $r = 0.54$, $P < 0.01$); B: Suspected Crohn's disease ($r = -0.40$); C: Polyposis plus carcinoid ($r = 0.56$, $P < 0.01$); D: Other indications ($r = 0.90$, $P < 0.01$), shown by the black line. The trend of this correlation is shown by the red line. DY: Diagnostic yield; SBTT: Small bowel transit time.

bust. If erythromycin mainly influences GTT, it might be an interesting prokinetic agent to use prior to small bowel CE.

Other prokinetics that have been studied in CE are metoclopramide and mosapride. Both prokinetic agents accelerated GTT and increased capsule completion rates, but had no influence on SBTT[26,27]. In our study, we used domperidone as a prokinetic agent in the majority of patients. Domperidone has shown to be effective in treating diabetic gastroparesis[28], but there are no data on the use this agent in CE.

Another way to use prokinetics may be with the aid of a real-time viewer system. In this way, prokinetics (or water or additional PEG) can be administered when the real-time viewer shows delayed gastric emptying. There are three studies that show a higher diagnostic yield of CE when a real-time viewer is used with on-demand administration of prokinetics, water, PEG or endoscopic-assisted duodenal placement[29-31].

The strength of this study is that this the first study that investigated the relation between diagnostic yield of CE and SBTT in a large study population. This allowed for a subgroup analysis for the different indications of CE. Another strong point of our study in our view is the use of an appropriate statistical method for determining the relation between diagnostic yield and SBTT by using a running mean.

A limitation of this study is that all patients in our population received a prokinetic agent, which might have changed the SBTT and with that also the diagnostic yield. The diagnostic yield might have been higher in this study population when we would not have used a prokinetic agent. Another limitation of this study is that during this study period we switched our prokinetic agent from domperidone to erythromycin. One should realize that this is a retrospective study. Prospective studies are necessary to establish the effect of the use of prokinetics on SBTT and diagnostic yield.

There may be many factors that influence SBTT and thereby diagnostic yield in small bowel CE. In this study we did not analyze such other factors, mainly because the main goal of this study was to determine whether there was a relation between diagnostic yield and SBTT at all. A previous study on the effect of SBTT on diagnostic yield found an independent association between diagnostic yield and SBTT[17]. In that study, no relation was found between diagnostic yield and other potential risk factors such as age, gender, study indication, hospital status, and

quality of bowel preparation[17]. Since we also found a positive correlation between diagnostic yield and SBTT it might be very interesting to look further into factors influencing SBTT and thereby diagnostic yield in future studies.

In conclusion, in this study with a large group of patients, we found a positive correlation between the diagnostic yield of small bowel CE and small bowel transit time for all indications except for suspected Crohn's disease. For clinical practice, these data implicate that it may not be advisable to use prokinetic agents which accelerate small bowel transit although this remains to be proven in future studies.

COMMENTS

Background

Capsule endoscopy (CE) is a very sensitive diagnostic technique to detect small bowel pathology. For a good and complete evaluation of the small bowel, the capsule should reach the cecum within recording time which is eight to eleven hours depending on the manufacturer. Some investigators advocate the use of a prokinetic agent to speed up the gastric and small bowel transit. However, a short bowel transit time (SBTT) may influence the diagnostic yield of CE. With colonoscopy, the detection rate of neoplastic lesions is higher when the time to withdraw the colonoscope is longer. It is conceivable that a similar principle also applies for small bowel CE. Therefore the question is whether the diagnostic yield of CE depends on the small bowel transit time.

Research frontiers

This the first study that investigated the relation between diagnostic yield of CE and SBTT in a large study population. This possible relation was also investigated for different indications for CE.

Innovations and breakthroughs

Six hundred and ninety-one consecutive CE procedures were analyzed. This study found a positive correlation between the diagnostic yield of CE and small bowel transit time, irrespective of whether the capsule had reached the cecum within recording time. These findings are in accordance with those from colonoscopy studies. This means that a longer transit time, implicating more images of the small bowel, resulted in a higher diagnostic yield. We found a positive correlation between the diagnostic yield and SBTT for all indications except for suspected Crohn's disease. The latter is probably due to the multiple and widespread small bowel lesions usually seen in Crohn's disease.

Applications

What does this positive correlation between diagnostic yield and SBTT mean for clinical practice? CE is less valuable when the cecum is not reached within recording time, but on the other hand our study indicates that the diagnostic yield is lower when the SBTT is shorter. So, ideally, the SBTT is long, but not so long that the capsule does not reach the cecum within recording time. One important issue in this matter is whether there is a role for prokinetic agents in CE to influence small bowel transit time. There is no consensus in the literature on this subject. For clinical practice, these data implicate that it may not be advisable to use prokinetic agents which accelerate small bowel transit, although this remains to be proven in future studies.

Peer review

It is a nice and interesting work. It values an important aspect of the capsule endoscopy not well studied until now: the relation between the diagnostic yield of capsule endoscopy and small bowel transit time.

REFERENCES

1 **Westerhof J**, Koornstra JJ, Weersma RK. Capsule endoscopy: a review from the clinician's perspectives. *Minerva Gastroenterol Dietol* 2008; **54**: 189-207

2 **Matsumoto T**, Esaki M, Moriyama T, Nakamura S, Iida M. Comparison of capsule endoscopy and enteroscopy with the double-balloon method in patients with obscure bleeding and polyposis. *Endoscopy* 2005; **37**: 827-832

3 **Hadithi M**, Heine GD, Jacobs MA, van Bodegraven AA, Mulder CJ. A prospective study comparing video capsule endoscopy with double-balloon enteroscopy in patients with obscure gastrointestinal bleeding. *Am J Gastroenterol* 2006; **101**: 52-57

4 **Nakamura M**, Niwa Y, Ohmiya N, Miyahara R, Ohashi A, Itoh A, Hirooka Y, Goto H. Preliminary comparison of capsule endoscopy and double-balloon enteroscopy in patients with suspected small-bowel bleeding. *Endoscopy* 2006; **38**: 59-66

5 **Ohmiya N**, Yano T, Yamamoto H, Arakawa D, Nakamura M, Honda W, Itoh A, Hirooka Y, Niwa Y, Maeda O, Ando T, Yao T, Matsui T, Iida M, Tanaka S, Chiba T, Sakamoto C, Sugano K, Goto H. Diagnosis and treatment of obscure GI bleeding at double balloon endoscopy. *Gastrointest Endosc* 2007; **66**: S72-S77

6 **Kaffes AJ**, Siah C, Koo JH. Clinical outcomes after double-balloon enteroscopy in patients with obscure GI bleeding and a positive capsule endoscopy. *Gastrointest Endosc* 2007; **66**: 304-309

7 **Fujimori S**, Seo T, Gudis K, Tanaka S, Mitsui K, Kobayashi T, Ehara A, Yonezawa M, Tatsuguchi A, Sakamoto C. Diagnosis and treatment of obscure gastrointestinal bleeding using combined capsule endoscopy and double balloon endoscopy: 1-year follow-up study. *Endoscopy* 2007; **39**: 1053-1058

8 **Kameda N**, Higuchi K, Shiba M, Machida H, Okazaki H, Yamagami H, Tanigawa T, Watanabe K, Watanabe T, Tominaga K, Fujiwara Y, Oshitani N, Arakawa T. A prospective, single-blind trial comparing wireless capsule endoscopy and double-balloon enteroscopy in patients with obscure gastrointestinal bleeding. *J Gastroenterol* 2008; **43**: 434-440

9 **Kamalaporn P**, Cho S, Basset N, Cirocco M, May G, Kortan P, Kandel G, Marcon N. Double-balloon enteroscopy following capsule endoscopy in the management of obscure gastrointestinal bleeding: outcome of a combined approach. *Can J Gastroenterol* 2008; **22**: 491-495

10 **Arakawa D**, Ohmiya N, Nakamura M, Honda W, Shirai O, Itoh A, Hirooka Y, Niwa Y, Maeda O, Ando T, Goto H. Outcome after enteroscopy for patients with obscure GI bleeding: diagnostic comparison between double-balloon endoscopy and videocapsule endoscopy. *Gastrointest Endosc* 2009; **69**: 866-874

11 **Niv Y**, Niv G, Wiser K, Demarco DC. Capsule endoscopy - comparison of two strategies of bowel preparation. *Aliment Pharmacol Ther* 2005; **22**: 957-962

12 **Westerhof J**, Weersma RK, Koornstra JJ. Risk factors for incomplete small-bowel capsule endoscopy. *Gastrointest Endosc* 2009; **69**: 74-80

13 **Benson ME**, Reichelderfer M, Said A, Gaumnitz EA, Pfau PR. Variation in colonoscopic technique and adenoma detection rates at an academic gastroenterology unit. *Dig Dis Sci* 2010; **55**: 166-171

14 **Barclay RL**, Vicari JJ, Doughty AS, Johanson JF, Greenlaw RL. Colonoscopic withdrawal times and adenoma detection during screening colonoscopy. *N Engl J Med* 2006; **355**: 2533-2541

15 **Barclay RL**, Vicari JJ, Greenlaw RL. Effect of a time-dependent colonoscopic withdrawal protocol on adenoma detection during screening colonoscopy. *Clin Gastroenterol Hepatol* 2008; **6**: 1091-1098

16 **Korman LY**, Delvaux M, Gay G, Hagenmuller F, Keuchel M, Friedman S, Weinstein M, Shetzline M, Cave D, de Franchis R. Capsule endoscopy structured terminology (CEST): proposal of a standardized and structured terminology for reporting capsule endoscopy procedures. *Endoscopy* 2005; **37**: 951-959

17 **Buscaglia JM**, Kapoor S, Clarke JO, Bucobo JC, Giday SA, Magno P, Yong E, Mullin GE. Enhanced diagnostic yield with prolonged small bowel transit time during capsule en-

doscopy. *Int J Med Sci* 2008; **5**: 303-308
18 **Fireman Z**, Kopelman Y, Friedman S, Ephrath H, Choman E, Debby H, Eliakim R. Age and indication for referral to capsule endoscopy significantly affect small bowel transit times: the given database. *Dig Dis Sci* 2007; **52**: 2884-2887
19 **Prather CM**, Camilleri M, Thomforde GM, Forstrom LA, Zinsmeister AR. Gastric axial forces in experimentally delayed and accelerated gastric emptying. *Am J Physiol* 1993; **264**: G928-G934
20 **Keshavarzian A**, Isaac RM. Erythromycin accelerates gastric emptying of indigestible solids and transpyloric migration of the tip of an enteral feeding tube in fasting and fed states. *Am J Gastroenterol* 1993; **88**: 193-197
21 **Bruley des Varannes S**, Parys V, Ropert A, Chayvialle JA, Rozé C, Galmiche JP. Erythromycin enhances fasting and postprandial proximal gastric tone in humans. *Gastroenterology* 1995; **109**: 32-39
22 **Niv E**, Bonger I, Barkay O, Halpern Z, Mahajna E, Depsames R, Kopelman Y, Fireman Z. Effect of erythromycin on image quality and transit time of capsule endoscopy: a two-center study. *World J Gastroenterol* 2008; **14**: 2561-2565
23 **Leung WK**, Chan FK, Fung SS, Wong MY, Sung JJ. Effect of oral erythromycin on gastric and small bowel transit time of capsule endoscopy. *World J Gastroenterol* 2005; **11**: 4865-4868
24 **Fireman Z**, Paz D, Kopelman Y. Capsule endoscopy: improving transit time and image view. *World J Gastroenterol* 2005; **11**: 5863-5866
25 **Caddy GR**, Moran L, Chong AK, Miller AM, Taylor AC, Desmond PV. The effect of erythromycin on video capsule endoscopy intestinal-transit time. *Gastrointest Endosc* 2006; **63**: 262-266
26 **Wei W**, Ge ZZ, Lu H, Gao YJ, Hu YB, Xiao SD. Effect of mosapride on gastrointestinal transit time and diagnostic yield of capsule endoscopy. *J Gastroenterol Hepatol* 2007; **22**: 1605-1608
27 **Selby W**. Complete small-bowel transit in patients undergoing capsule endoscopy: determining factors and improvement with metoclopramide. *Gastrointest Endosc* 2005; **61**: 80-85
28 **Sugumar A**, Singh A, Pasricha PJ. A systematic review of the efficacy of domperidone for the treatment of diabetic gastroparesis. *Clin Gastroenterol Hepatol* 2008; **6**: 726-733
29 **Ogata H**, Kumai K, Imaeda H, Aiura K, Hisamatsu T, Okamoto S, Iwao Y, Sugino Y, Kitajima M, Hibi T. Clinical impact of a newly developed capsule endoscope: usefulness of a real-time image viewer for gastric transit abnormality. *J Gastroenterol* 2008; **43**: 186-192
30 **Hosono K**, Endo H, Sakai E, Sekino Y, Uchiyama T, Watanabe S, Iida H, Sakamoto Y, Koide T, Takahashi H, Yoneda M, Tokoro C, Abe Y, Inamori M, Kobayashi N, Kubota K, Nakajima A. Optimal approach for small bowel capsule endoscopy using polyethylene glycol and metoclopramide with the assistance of a real-time viewer. *Digestion* 2011; **84**: 119-125
31 **Shiotani A**, Honda K, Kawakami M, Nishi R, Murao T, Ishii M, Matsumoto H, Kusunoki H, Hata J, Haruma K. Use of an external real-time image viewer coupled with prespecified actions enhanced the complete examinations for capsule endoscopy. *J Gastroenterol Hepatol* 2011; **26**: 1270-1274

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BRIEF ARTICLE

Identification of individuals with non-alcoholic fatty liver disease by the diagnostic criteria for the metabolic syndrome

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Abstract

AIM: To clarify the efficiency of the criterion of metabolic syndrome to detecting non-alcoholic fatty liver disease (NAFLD).

METHODS: Authors performed a cross-sectional study involving participants of a medical health checkup program including abdominal ultrasonography. This study involved 11 714 apparently healthy Japanese men and women, 18 to 83 years of age. NAFLD was defined by abdominal ultrasonography without an alcohol intake of more than 20 g/d, known liver disease, or current use of medication. The revised criteria of the National Cholesterol Education Program Adult Treatment Panel Ⅲ were used to characterize the metabolic syndrome.

RESULTS: NAFLD was detected in 32.2% (95% CI: 31.0%-33.5%) of men (n = 1874 of 5811) and in 8.7% (95% CI: 8.0%-9.5%) of women (n = 514 of 5903). Among obese people, the prevalence of NAFLD was as high as 67.3% (95% CI: 64.8%-69.7%) in men and 45.8% (95% CI: 41.7%-50.0%) in women. Although NAFLD was thought of as being the liver phenotype of metabolic syndrome, the prevalence of the metabolic syndrome among subjects with NAFLD was low both in men and women. 66.8% of men and 70.4% of women with NAFLD were not diagnosed with the metabolic syndrome. 48.2% of men with NAFLD and 49.8% of women with NAFLD weren't overweight [body mass index (BMI) $\geqslant$ 25 kg/m^2]. In the same way, 68.6% of men with NAFLD and 37.9% of women with NAFLD weren't satisfied with abdominal classification ($\geqslant$ 90 cm for men and $\geqslant$ 80 cm for women). Next, authors defined it as positive at screening for NAFLD when participants satisfied at least one criterion of metabolic syndrome. The sensitivity of the definition "at least 1 criterion" was as good as 84.8% in men and 86.6% in women. Separating subjects by BMI, the sensitivity was higher in obese men and women than in non-obese men and women (92.3% *vs* 76.8% in men, 96.1% *vs* 77.0% in women, respectively).

CONCLUSION: Authors could determine NAFLD effectively in epidemiological study by modifying the usage of the criteria for metabolic syndrome.

Key words: Nonalcoholic fatty liver; Metabolic syndrome; Population based study; Methodology

Peer reviewer: Liqing Yu, Assistant Professor, Pathology-Lipid Sciences, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem 27157-1040, United States

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a common clinical condition with histological features that resemble those of alcohol-induced liver injury, but occurs in patients who do not drink an excessive amount of alcohol (ethanol > 20 g/d)[1,2]. This disease is often associated with obesity[3], type 2 diabetes mellitus[4,5], dyslipidemia[6], and hypertension[7]. Each of these abnormalities carries a cardiovascular disease risk, and together they are often categorized as the insulin resistance syndrome or the metabolic syndrome[8-15].

NAFLD is now considered to be the hepatic representation of the metabolic syndrome[10-15].

Conventional radiology studies used in the diagnosis of fatty liver include ultrasound (US), computed tomography, and magnetic resonance (MR) imaging. Other than these radiological studies, we have no sensitive and low invasive screening method for NAFLD. Alanine aminotransferase (ALT) > 30 IU/L was usually used as the cut off level of screening NAFLD[16,17]. This threshold had a sensitivity of 0.92 for detecting the fatty-fibrotic pattern proven by ultrasound among obese children[18]. However, ALT was within normal levels in 69% of those who had increased liver fat[19]. Similarly, in the Dallas Heart Study, 79% of the subjects with a fatty liver (liver fat content > 5.6%) had normal serum ALT[20]. This implies that a normal ALT does not exclude steatosis. Aspartate aminotransferase (AST) and gamma glutamyltransferase (GGT) also correlate with liver fat content independent of obesity[21], but are even less sensitive than serum ALT.

It was well known that NAFLD was associated with the metabolic syndrome and patients with NAFLD tend to be accompanied with the abnormal component of the metabolic syndrome. However, the efficiency of the criterion of metabolic syndrome for detecting NAFLD has not yet been clarified. We aimed to clarify the efficiency and perform a cross sectional study among apparent healthy Japanese.

MATERIALS AND METHODS

Study design

We performed a cross-sectional study involving participants of a medical health checkup program including abdominal ultrasonography. The program was conducted in the Medical Health Checkup Center at Murakami Memorial Hospital, Gifu, Japan. The purpose of the medical health checkup program is to promote public health through early detection of chronic diseases and the evaluation of their underlying risk factors. Known as a "human dock", medical services of this kind are very popular in Japan.

Study population

All the subjects participating in such health checkup programs at Murakami Memorial Hospital between January 2004 and December 2008 were invited to join this study. The study was approved by the ethics committee of Murakami Memorial Hospital.

Data collection and exclusion criteria were described previously[8]. In short, we collected the data from urinalysis, blood cell counts, blood chemistry and abdominal ultrasonography. The medical history and lifestyle factors were collected by using a self-administered questionnaire. Exclusion criteria were an alcohol intake of more than 20 g/d, known liver disease, or current use of medication which could influence the metabolic syndrome such as anti-diabetic drugs, anti-hypertensive drugs, anti-dyslipidemic drugs, anti-gout drugs, and/or anti-obesity drugs[8,10].

According to the revised National Cholesterol Education Program Adult Treatment Panel Ⅲ (NCEP-ATP Ⅲ)[22] or the new International Diabetes Federation (IDF) definition[23], subjects who had three or more of the following criteria were diagnosed as having the metabolic syndrome. Fatty liver was defined on the basis of ultrasonographic findings[24]. Of 4 known criteria (hepatorenal echo contrast, liver brightness, deep attenuation, and vascular blurring), the participants were required to have hepatorenal contrast and liver brightness to be given a diagnosis of fatty liver[24].

During study period, we invited 20 012 participants in the health checkup program to enroll in the study. Of those, a total of 17 262 Japanese participants (10 329 men and 6933 women) were enrolled after giving informed consent to be included in the study. We excluded 621 participants (420 men and 201 women) who had known liver disease. In addition, 3330 participants (3042 men and 288 women) who consumed more than 20 g of ethanol per day and 1579 participants (1056 men and 541 women) who were currently receiving medication were excluded. As a result, this study ultimately consisted of 11 714 participants (5811 men and 5903 women). The mean ± SD age was 45.5 ± 9.4 years (range: 18 years to 83 years) for men and 44.3 ± 9.3 years (range: 18 years to 79 years) for women, respectively. The mean body mass index (BMI) was 23.2 ± 3.1 kg/m^2 (range: 14.3 to 41.0 kg/m^2) in men and 21.1 ± 3.0 kg/m^2 (range: 14.0 to 58.3 kg/m^2) in women, respectively. The mean abdominal circumference was 81.2 ± 8.1 cm (range: 57.3 cm to 127.5 cm) in men and 71.4 ± 8.2 cm (range: 49.0 cm to 145.0 cm) in women, respectively.

Table 1 The basic characteristics of the study population and the association of nonalcoholic fatty liver disease with gender difference

Men	Total *n* (%)	Obese *n* (%)	Non-obese *n* (%)
Number	5811	1441	4370
NAFLD	1874 (32.2)	970 (67.3)	904 (20.7%)
5 criteria of the metabolic syndrome			
Increased abdominal circumference	791 (13.6)	703 (48.8)	88 (2)
Elevated fasting glucose level	1967 (33.8)	704 (48.9)	1263 (28.9)
Elevated blood pressure	1294 (22.3)	575 (39.9)	719 (16.5)
Decreased HDL cholesterol level	1736 (29.9)	654 (45.4)	1082 (24.8)
Elevated triglyceride level	1063 (18.3)	484 (33.6)	579 (13.2)
ALT > 30	1269 (21.8)	670 (46.5)	599 (13.7)
MS defined by rNCEP-ATP Ⅲ	873 (15)	578 (40.1)	295 (6.8)
MS defined by IDF	479 (8.2)	443 (30.7)	36 (0.8)
At least 1 criterion	3680 (63.3)	1291 (89.6)	2389 (54.7)
At least 2 criteria	1955 (33.6)	957 (66.4)	998 (22.8)
At least 1 criterion or ALT > 30 IU/L	3885 (66.9)	1337 (92.8)	2548 (58.3)
Women			
Number	5903	563	5340
NAFLD	514 (8.7)	258 (45.8)	256 (4.8)
5 criteria of the metabolic syndrome			
Increased abdominal circumference	878 (14.9)	430 (76.4)	448 (8.4)
Elevated fasting glucose level	679 (11.5)	176 (31.3)	503 (9.4)
Elevated blood pressure	578 (9.8)	185 (32.9)	393 (7.4)
Decreased HDL cholesterol level	1320 (22.4)	265 (47.1)	1055 (19.8)
Elevated triglyceride level	195 (3.3)	73 (13)	122 (2.3)
Elevated ALT (ALT > 30 IU/L)	200 (3.4)	78 (13.9)	122 (2.3)
MS defined by rNCEP-ATP Ⅲ	300 (5.1)	174 (30.9)	126 (2.4)
MS defined by IDF	254 (4.3)	162 (28.8)	92 (1.7)
At least 1 criterion	2374 (40.2)	511 (90.8)	1863 (34.9)
At least 2 criteria	853 (14.5)	355 (63.1)	498 (9.3)
At least 1 criterion or elevated ALT	2430 (41.2)	515 (91.5)	1915 (35.9)

NAFLD: Nonalcoholic fatty liver disease; US: Abdominal ultrasonography; BMI: Body mass index; HDL: High density lipoprotein; MS: Metabolic syndrome; rNCEP-ATPⅢ: Revised National Cholesterol Education Program Adult Treatment Panel Ⅲ definition; IDF: International diabetes federation definition; ALT: Alanine aminotransferase.

Statistical analysis

The R version 2.9.0 (available from http://www.r-project.org/) was used for statistical analyses. Two groups of subjects were compared by using the unpaired *t*-test and the chi-square test, and a $P < 0.05$ was accepted as a significant level.

RESULTS

Basic characteristics of study population

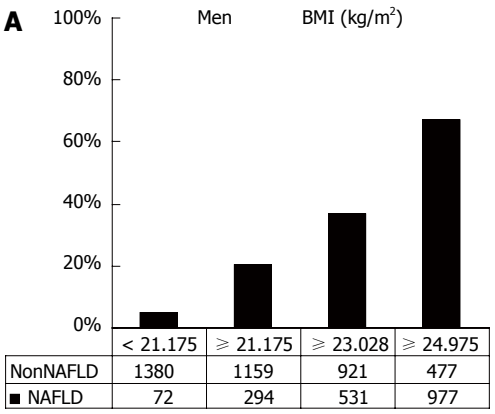
The metabolic syndrome defined by revised NCEP-ATPⅢ definition was detected in 15.0% (95% CI: 14.1%-16.0%) of men ($n = 873$ of 5811) and in 5.1% (95% CI: 4.5%-5.7%) of women ($n = 300$ of 5903). The metabolic syndrome defined by IDF definition was detected in 8.2% (95% CI: 7.5%-9.0%) of men ($n = 479$ of 5811) and in 4.3% (95% CI: 3.8%-4.8%) of women ($n = 254$ of 5903) (Table 1). Among obese people, the metabolic syndrome defined by revised NCEP-ATPⅢ definition was detected in 40.1% (95% CI: 37.6%-42.7%) of men and in 30.9% (95% CI: 27.1%-34.9%) of women, and the metabolic syndrome defined by IDF definition was detected in 30.7% (95% CI: 28.4%-33.2%) of men and in 28.8% (95% CI: 25.1%-32.7%) of women, respectively (Table 1).

Association of NAFLD with gender difference, or body fat accumulation

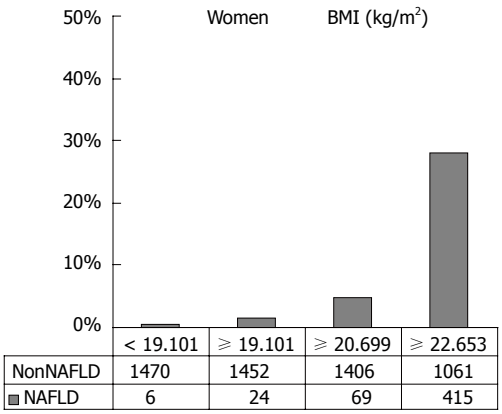
NAFLD was detected in 32.2% (95% CI: 31.0%-33.5%) of men ($n = 1874$ of 5811) and in 8.7% (95% CI: 8.0%-9.5%) of women ($n = 514$ of 5903). The prevalence of NAFLD in men was four times higher than those in women (Table 1). Among obese people, the prevalence of NAFLD was as high as 67.3% (95% CI: 64.8%-69.7%) in men and 45.8% (95% CI: 41.7%-50.0%) in women (Table 1). NAFLD was associated with body fat accumulation strongly both in men and women.

When we separated by quartile the subjects according to their BMI or abdominal circumference, half of NAFLD men and three quarters of NAFLD women were classified in the superior quartile. The prevalence of NAFLD was increased according to the increase of BMI or abdominal circumference (Figure 1A). The role of BMI for NAFLD was equal to that of abdominal circumference both in men and women. The ratio of NAFLD in the superior quartile/total NAFLD was higher in women than in men. The prevalence of individuals who met two or more of the MS criteria other than waist circumference was increased according to the increase of BMI or abdominal circumference (Figure 1B).

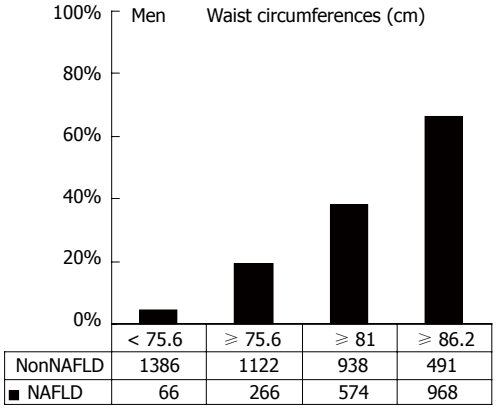
A



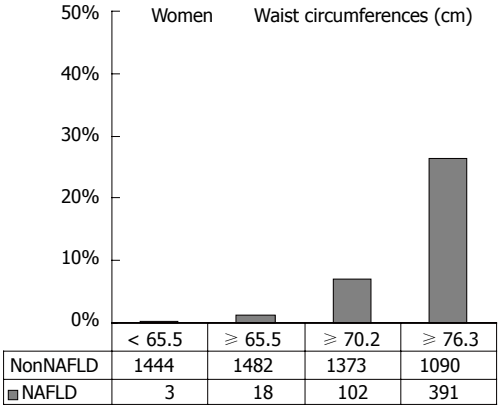
	< 21.175	⩾ 21.175	⩾ 23.028	⩾ 24.975
NonNAFLD	1380	1159	921	477
■ NAFLD	72	294	531	977



	< 19.101	⩾ 19.101	⩾ 20.699	⩾ 22.653
NonNAFLD	1470	1452	1406	1061
■ NAFLD	6	24	69	415

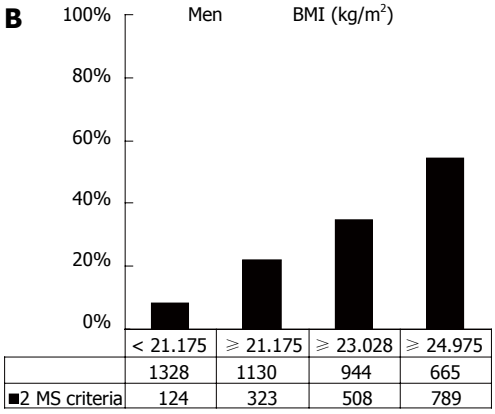


	< 75.6	⩾ 75.6	⩾ 81	⩾ 86.2
NonNAFLD	1386	1122	938	491
■ NAFLD	66	266	574	968

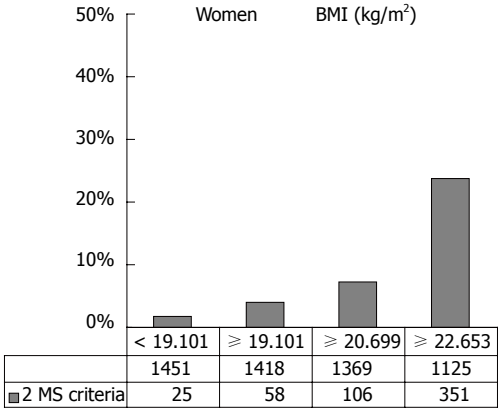


	< 65.5	⩾ 65.5	⩾ 70.2	⩾ 76.3
NonNAFLD	1444	1482	1373	1090
■ NAFLD	3	18	102	391

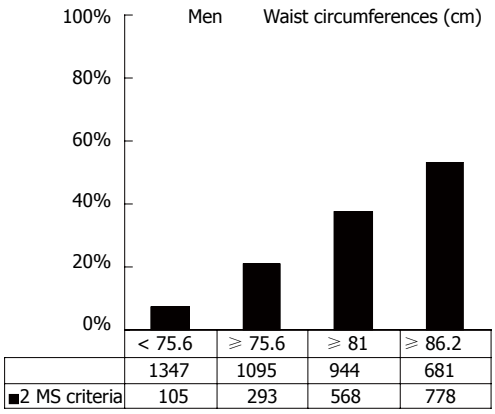
B



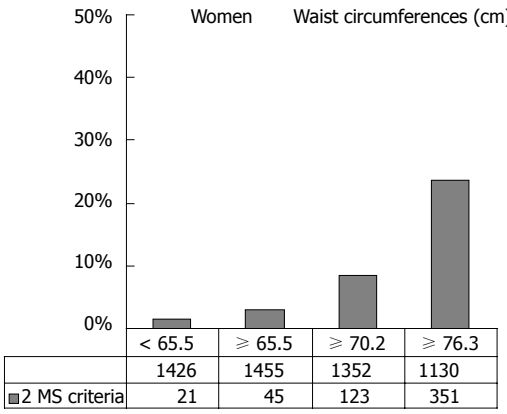
	< 21.175	⩾ 21.175	⩾ 23.028	⩾ 24.975
	1328	1130	944	665
■2 MS criteria	124	323	508	789



	< 19.101	⩾ 19.101	⩾ 20.699	⩾ 22.653
	1451	1418	1369	1125
■2 MS criteria	25	58	106	351



	< 75.6	⩾ 75.6	⩾ 81	⩾ 86.2
	1347	1095	944	681
■2 MS criteria	105	293	568	778



	< 65.5	⩾ 65.5	⩾ 70.2	⩾ 76.3
	1426	1455	1352	1130
■2 MS criteria	21	45	123	351

Figure 1 We separated the subjects by quartile according to their body mass index or abdominal circumference. A: The bar indicated the prevalence (%) of individuals with NAFLD; B: Individuals who meet two or more of the MS criteria other than waist circumference according to BMI or waist circumference quartiles. 2 MS criteria means individuals who meets two or more of the MS criteria other than waist circumference. NAFLD: Nonalcoholic fatty liver disease; BMI: Body mass index; MS: Metabolic syndrome.

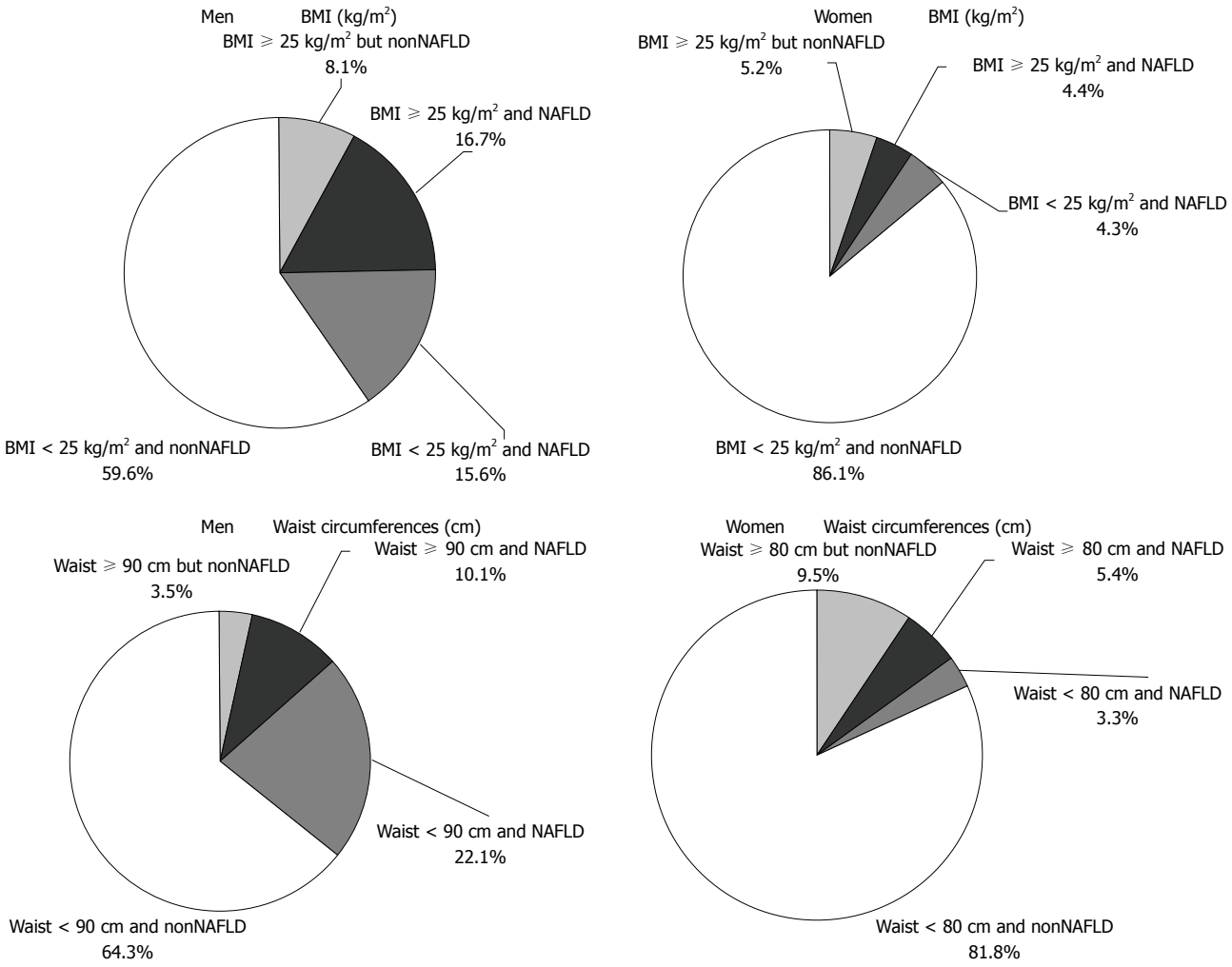


Figure 2 This figure indicates the prevalence of non-alcoholic fatty liver disease and alcoholic fatty liver disease with or without patients being overweight (BMI ⩾ 25 kg/m²) or having elevated abdominal circumferences (⩾ 90 cm for men and ⩾ 80 cm for women). Data was expressed as prevalence (%). NAFLD accompanied with being overweight occurred in 51.8% of NAFLD men (970/1874) and 50.2% of NAFLD women (258/514). NAFLD accompanied by elevated abdominal circumference occurred in 31.4% of NAFLD men (588/1874) and 62.1% of women (319/514). NAFLD: Non-alcoholic fatty liver disease; BMI: Body mass index.

Role of the criteria of the metabolic syndrome in detecting or diagnosing NAFLD in obese or non-obese population

Although NAFLD was associated with obesity or body fat accumulation strongly, the population that was neither overweight (BMI ⩾ 25 kg/m^2) nor had elevated abdominal circumference was not small (Figure 2). Actually, 48.2% of men with NAFLD and 49.8% of women with NAFLD were not overweight (BMI ⩾ 25 kg/m^2). Similarly, 68.6% of men with NAFLD and 37.9% of women with NAFLD did not satisfy increased abdominal circumference classification. Half of the NAFLD group was classified as non-obese, but the prevalence of NAFLD among the non-obese population was lower. These facts means an effective method is needed to detect NAFLD among the non-obese population. Then, we separated the subjects into two groups, obese group or non-obese group, and investigated the efficacy of the criteria of metabolic syndrome for detecting NAFLD in each group.

Among the criteria for metabolic syndrome, the criterion of abdominal circumferences (⩾ 80 cm) had high sensitivity (87.6%) for detecting NAFLD in women who were overweight (BMI ⩾ 25 kg/m^2) (Table 2). In other words, abdominal circumference was effective for detecting NAFLD in obese women. However, the criterion of abdominal circumference had low sensitivity (36.3%) in non-obese women. The sensitivity of abdominal circumference (⩾ 90 cm) was very low (5.8%) in non-obese men. Even in obese men the sensitivity was not high (55.3%). Other criteria for metabolic syndrome had higher sensitivity in obese men and women than in the non-obese population but sensitivity never exceeded 60%.

As a screening tool for NAFLD, the sensitivity of elevated ALT (ALT > 30 IU/L) was 49.7% in men, which exceeded the sensitivity of the criteria of metabolic syndrome, but it was 17.7% in women, which was lower than all metabolic syndrome criteria were. On the other hand, the specificity of elevated ALT was as high as 90.6% in men and 98.0% in women, but the criteria of metabolic syndrome had equally high specificity.

Next, we defined it as positive at screening for NAFLD

Table 2 The role of the criteria of the metabolic syndrome in detecting or diagnosing nonalcoholic fatty liver disease in obese or non-obese population

	Men				Women			
Sensitivity	Total %	Obese %	Non-obese %	*P* value	Total %	Obese %	Non-obese %	*P* value
5 criteria of the metabolic syndrome								
Increased abdominal circumference	31.40	55.30	5.80	< 0.001	62.10	87.60	36.30	< 0.001
Elevated fasting glucose level	49.10	52.10	45.90	0.008	36.80	42.20	31.30	0.013
Elevated blood pressure	34.70	44.10	24.60	< 0.001	31.90	41.50	22.30	< 0.001
Decreased HDL cholesterol level	44.10	49.10	38.80	< 0.001	50.40	56.60	44.10	0.006
Elevated triglyceride level	35.20	41.00	28.90	< 0.001	17.90	20.50	15.20	0.15
Elevated ALT (ALT > 30 IU/L)	47.90	59.20	35.80	< 0.001	17.70	24.00	11.30	< 0.001
MS defined by rNCEP-ATP Ⅲ	33.20	48.60	16.80	< 0.001	32.50	45.00	19.90	< 0.001
MS defined by IDF	21.00	38.10	2.70	< 0.001	29.60	43.40	15.60	< 0.001
At least 1 criterion	84.80	92.30	76.80	< 0.001	86.60	96.10	77.00	< 0.001
At least 2 criteria	61.00	74.20	46.90	< 0.001	61.10	77.50	44.50	< 0.001
At least 1 criterion or elevated ALT	90.40	96.20	84.20	< 0.001	87.40	96.90	79.70	< 0.001
Specificity								
5 criteria of the metabolic syndrome								
Increased abdominal circumference	94.80	64.50	99.00	< 0.001	89.60	33.10	93.00	< 0.001
Elevated fasting glucose level	73.40	57.70	75.50	< 0.001	90.90	78.00	91.70	< 0.001
Elevated blood pressure	83.60	68.80	85.70	< 0.001	92.30	74.40	93.40	< 0.001
Decreased HDL cholesterol level	76.90	62.20	78.90	< 0.001	80.30	61.00	81.50	< 0.001
Elevated triglyceride level	89.70	81.70	90.80	< 0.001	98.10	93.40	98.40	< 0.001
Elevated ALT (ALT > 30 IU/L)	90.60	79.60	92.10	< 0.001	98.00	94.80	98.20	< 0.001
MS defined by rNCEP-ATP Ⅲ	93.60	77.30	95.90	< 0.001	97.50	81.00	98.50	< 0.001
MS defined by IDF	97.80	84.50	99.70	< 0.001	98.10	83.60	99.00	< 0.001
At least 1 criterion	46.90	15.90	51.10	< 0.001	64.20	13.80	67.20	< 0.001
At least 2 criteria	79.40	49.70	83.40	< 0.001	90.00	49.20	92.40	< 0.001
At least 1 criterion or elevated ALT	44.30	14.20	48.40	< 0.001	63.20	13.10	65.40	< 0.001

NAFLD: Nonalcoholic fatty liver disease; US: Abdominal ultrasonography; BMI: Body mass index; HDL: High dense lipoprotein; MS: Metabolic syndrome; rNCEP-ATPⅢ: Revised National Cholesterol Education Program Adult Treatment Panel Ⅲ definition; IDF: International Diabetes Federation definition; ALT: Alanine aminotransferase.

when participants satisfied at least one or two components of metabolic syndrome. The sensitivity of the definition "at least 1 criterion" was 84.8% in men and 86.6% in women. Separating subjects with BMI, the sensitivity was higher in obese men and women than in non-obese men and women (92.3% *vs* 76.8% in men, 96.1% *vs* 77.0% in women, respectively).

The prevalence of subjects with NAFLD who also had the metabolic syndrome is indicated in Figure 3. Although NAFLD was thought of as being the liver phenotype of metabolic syndrome, the prevalence of the metabolic syndrome among subjects with NAFLD was low both in men and women. Among men with NAFLD, 66.8% were not diagnosed with the metabolic syndrome defined by revised NCEP-ATPⅢ definition, and 79.0% were not diagnosed with the metabolic syndrome as defined by revised IDF definition. Even in women, 70.4% and 67.5%, respectively, were not diagnosed withmetabolic syndrome by revised NCEP-ATPⅢ definition and revised IDF definition. These results mean that a large number of participants diagnosed with the metabolic syndrome have NAFLD, but a large number of participants with NAFLD were not diagnosed with the metabolic syndrome, whether we used revised NECP-ATPⅢ criteria or IDF criteria.

DISCUSSION

In this study, we clarified the impact of the criteria of the metabolic syndrome for diagnosing NAFLD in a healthy population. The metabolic syndrome was associated with abdominal obesity and its criteria include waist circumference[22,23,25,26], and NAFLD was reported to be associated with abdominal obesity. However, our results indicated there was no significant difference between BMI and waist circumferences as the strength of association with NAFLD or the accumulation of metabolic syndrome criteria.

The presence of multiple metabolic disorders such as diabetes mellitus, obesity, dyslipidaemia and hypertension is associated with a potentially progressive, severe liver disease[15,27]. Previous reports demonstrated that prevalence of NAFLD increased to 10%-80% in individuals with obesity, 35%-90% in individuals with type 2 diabetes mellitus, 30%-56% in individuals with hypertension, and 26%-58% in individuals with dyslipidemia[9,28-30]. Another study in a Japanese population showed that prevalence of NAFLD increased to 43% in individuals with impaired fasting glucose and 62% in individuals with type 2 diabetes mellitus[28]. Some studies estimate the prevalence of NAFLD be up to 15%-30% of the general population[8,31,32], and the prevalence of metabolic syndrome was estimated to be up to 25% of the general population[33]. In those patients with the metabolic syndrome, liver fat content is significantly increased up to 4-fold higher than those without the metabolic syndrome[34], and the incidence of NAFLD has been shown to be increased 4-fold in men and 11-fold in women with the metabolic syndrome[8].

Our data clearly indicated that 21% to 33% of sub-

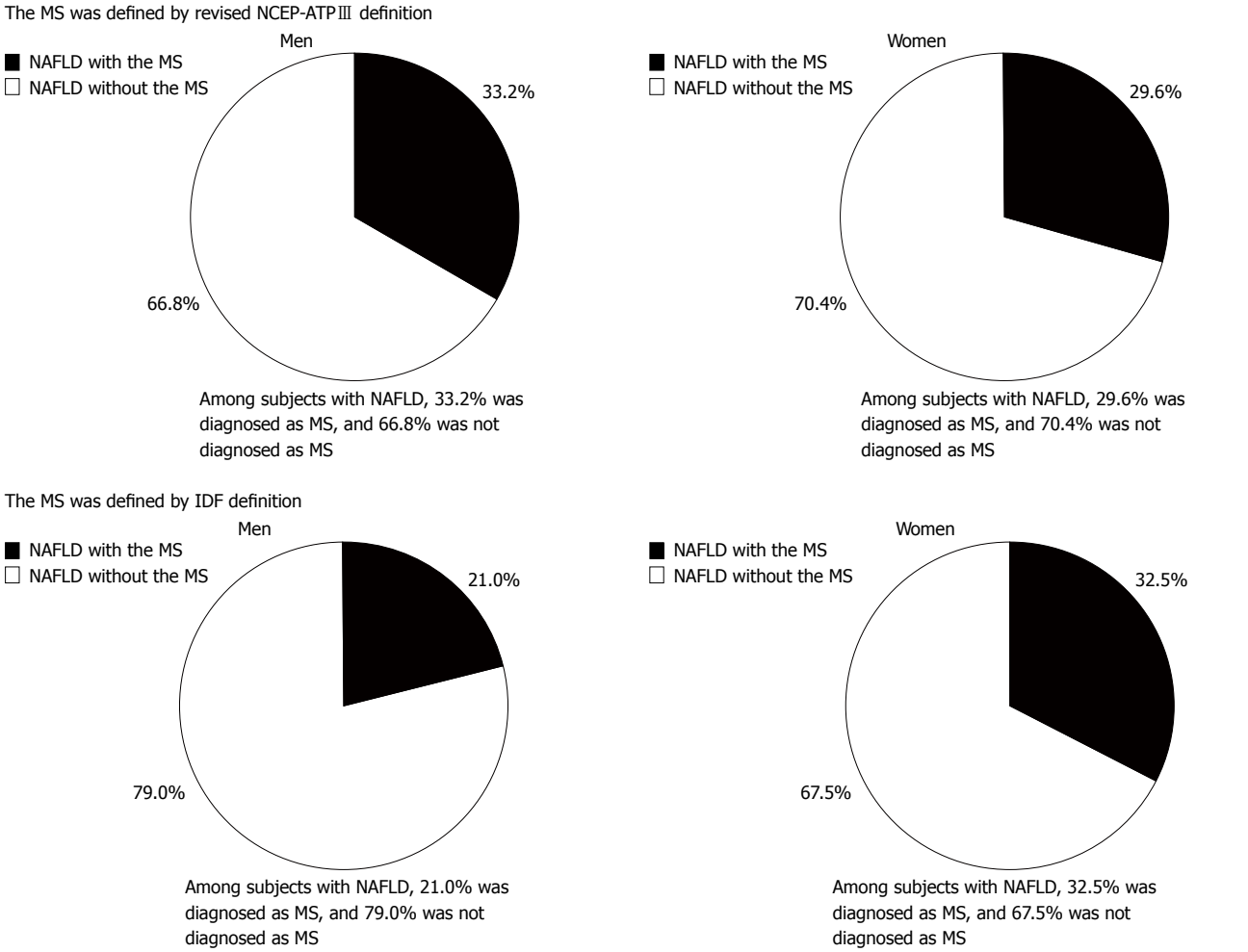


Figure 3 The prevalence of subjects with or without the metabolic syndrome among 1874 men and 514 women with non-alcoholic fatty liver disease. Data was expressed as prevalence (%). The metabolic syndrome (MS) was diagnosed using revised IDF. Among men with NAFLD, 66.8% and 79.0% were not diagnosed with the MS defined by revised NCEP-ATPⅢ definition and revised IDF definition, respectively. In women, 70.4% and 67.5%, respectively, were not diagnosed with the MS by revised NCEP-ATPⅢ definition and revised IDF definition. IDF: International Diabetes Federation; NCEP-ATPⅢ: National Cholesterol Education Program Adult Treatment Panel Ⅲ; NAFLD: Non-alcoholic fatty liver disease.

jects with NAFLD, depending on gender and the criteria used, were diagnosed with the metabolic syndrome. Several previous studies reported how many subjects with NAFLD were diagnosed with the metabolic syndrome, but almost all previous studies were hospital studies. Three population based studies mentioned the prevalence of subjects with NAFLD who were diagnosed with the metabolic syndrome among the general population[8,35,36]. In these studies, the prevalence of the metabolic syndrome among subjects with NAFLD was 17% to 36% depending on gender and the criteria used. The reported prevalence was similar to ours.

There has been no report regarding the sensitivity and specificity of the metabolic syndrome for detecting NAFLD. Among the criteria for metabolic syndrome, the criterion of abdominal circumference had high sensitivity in obese women. However, it had low sensitivity (36.3%) in non-obese women and was very low (5.8%) in non-obese men and low (55.3% in obese men. Other than the criterion of abdominal circumference, none of the sensitivities exceeded 60%. In our study, the specificity of elevated ALT (ALT > 30 IU/L) was 90.6% in men and 98.0% in women. However, the sensitivity was as low as 47.9% in men and 17.7% in women. The specificity of elevated ALT was significantly higher among obese subjects than among non-obese subjects, and sensitivity was higher among obese subjects than among non-obese subjects.

When we investigated the predictability of each component of metabolic syndrome such as abdominal circumference, fasting blood sugar, serum lipid, and blood pressure, each component had high specificity but low sensitivity, similar to elevated ALT. Therefore, we defined it as screening positive for NAFLD, when subjects satisfied at least one criterion of metabolic syndrome; the sensitivity was 84.8% in men and 86.6% in women. Additionally, we defined it as positive when subjects satisfied at least one criterion of metabolic syndrome or elevated ALT. The sensitivity of "at least 1 criterion or elevated ALT" was 90.4% in men and 87.4% in women. However, the specificity of "at least 1 criterion or elevated ALT" was lower -44%-63%.

The result of our study means that we could identify NAFLD effectively in epidemiological study by modifying the usage of the criteria for metabolic syndrome. It is clinically critical evidence that a large part of patients with NAFLD were not diagnosed with the metabolic syndrome, when we used today's definition for the metabolic syndrome. However, our subject population consisted only of Japanese, thus, the generalizability of our study to non-Japanese populations is uncertain. It is one of our study limitations that we used abdominal ultrasonography for diagnosing NAFLD, although the validation ultrasonography had a sensitivity of 91.7% and a specificity of 100%[24].

COMMENTS

Background

It is well known that non-alcoholic fatty liver disease (NAFLD) is associated with the metabolic syndrome and patients with NAFLD tend to also have the metabolic syndrome.

Research frontiers

The impact of overlap between NAFLD and the metabolic syndrome has not been evaluated yet.

Innovations and breakthroughs

It is clinically critical evidence that a large number of patients with NAFLD were not diagnosed with the metabolic syndrome in a healthy Japanese population.

Applications

The authors could identify NAFLD effectively by modifying the usage of the criteria for metabolic syndrome.

Peer review

It is a relatively large population study. The conclusion is consistent with recent observations showing the dissociation between NAFLD and other parameters of metabolic syndrome. The readers of this journal will be interested in the findings of this study.

REFERENCES

1 **Brunt EM**, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999; **94**: 2467-2474

2 **Mulhall BP**, Ong JP, Younossi ZM. Non-alcoholic fatty liver disease: an overview. *J Gastroenterol Hepatol* 2002; **17**: 1136-1143

3 **Bellentani S**, Saccoccio G, Masutti F, Crocè LS, Brandi G, Sasso F, Cristanini G, Tiribelli C. Prevalence of and risk factors for hepatic steatosis in Northern Italy. *Ann Intern Med* 2000; **132**: 112-117

4 **Akbar DH**, Kawther AH. Nonalcoholic fatty liver disease in Saudi type 2 diabetic subjects attending a medical outpatient clinic: prevalence and general characteristics. *Diabetes Care* 2003; **26**: 3351-3352

5 **Gupte P**, Amarapurkar D, Agal S, Baijal R, Kulshrestha P, Pramanik S, Patel N, Madan A, Amarapurkar A. Non-alcoholic steatohepatitis in type 2 diabetes mellitus. *J Gastroenterol Hepatol* 2004; **19**: 854-858

6 **Assy N**, Kaita K, Mymin D, Levy C, Rosser B, Minuk G. Fatty infiltration of liver in hyperlipidemic patients. *Dig Dis Sci* 2000; **45**: 1929-1934

7 **Donati G**, Stagni B, Piscaglia F, Venturoli N, Morselli-Labate AM, Rasciti L, Bolondi L. Increased prevalence of fatty liver in arterial hypertensive patients with normal liver enzymes: role of insulin resistance. *Gut* 2004; **53**: 1020-1023

8 **Hamaguchi M**, Kojima T, Takeda N, Nakagawa T, Taniguchi H, Fujii K, Omatsu T, Nakajima T, Sarui H, Shimazaki M, Kato T, Okuda J, Ida K. The metabolic syndrome as a predictor of nonalcoholic fatty liver disease. *Ann Intern Med* 2005; **143**: 722-728

9 **Radu C**, Grigorescu M, Crisan D, Lupsor M, Constantin D, Dina L. Prevalence and associated risk factors of non-alcoholic fatty liver disease in hospitalized patients. *J Gastrointestin Liver Dis* 2008; **17**: 255-260

10 **McCullough AJ**. The clinical features, diagnosis and natural history of nonalcoholic fatty liver disease. *Clin Liver Dis* 2004; **8**: 521-533, viii

11 **Adams LA**, Angulo P. Recent concepts in non-alcoholic fatty liver disease. *Diabet Med* 2005; **22**: 1129-1133

12 **Marchesini G**, Marzocchi R, Agostini F, Bugianesi E. Nonalcoholic fatty liver disease and the metabolic syndrome. *Curr Opin Lipidol* 2005; **16**: 421-427

13 **Neuschwander-Tetri BA**. Nonalcoholic steatohepatitis and the metabolic syndrome. *Am J Med Sci* 2005; **330**: 326-335

14 **Lavine JE**, Schwimmer JB. Nonalcoholic fatty liver disease in the pediatric population. *Clin Liver Dis* 2004; **8**: 549-558, viii-ix

15 **Marchesini G**, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, Natale S, Vanni E, Villanova N, Melchionda N, Rizzetto M. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* 2003; **37**: 917-923

16 **Fraser A**, Longnecker MP, Lawlor DA. Prevalence of elevated alanine aminotransferase among US adolescents and associated factors: NHANES 1999-2004. *Gastroenterology* 2007; **133**: 1814-1820

17 **Strauss RS**, Barlow SE, Dietz WH. Prevalence of abnormal serum aminotransferase values in overweight and obese adolescents. *J Pediatr* 2000; **136**: 727-733

18 **Tazawa Y**, Noguchi H, Nishinomiya F, Takada G. Serum alanine aminotransferase activity in obese children. *Acta Paediatr* 1997; **86**: 238-241

19 **Szczepaniak LS**, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S, Hobbs HH, Dobbins RL. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. *Am J Physiol Endocrinol Metab* 2005; **288**: E462-E468

20 **Browning JD**, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, Grundy SM, Hobbs HH. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* 2004; **40**: 1387-1395

21 **Thamer C**, Tschritter O, Haap M, Shirkavand F, Machann J, Fritsche A, Schick F, Häring H, Stumvoll M. Elevated serum GGT concentrations predict reduced insulin sensitivity and increased intrahepatic lipids. *Horm Metab Res* 2005; **37**: 246-251

22 **Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults**. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA* 2001; **285**: 2486-2497

23 **Alberti KG**, Zimmet P, Shaw J. Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. *Diabet Med* 2006; **23**: 469-480

24 **Hamaguchi M**, Kojima T, Itoh Y, Harano Y, Fujii K, Nakajima T, Kato T, Takeda N, Okuda J, Ida K, Kawahito Y, Yoshikawa T, Okanoue T. The severity of ultrasonographic findings in nonalcoholic fatty liver disease reflects the metabolic syndrome and visceral fat accumulation. *Am J Gastroenterol* 2007; **102**: 2708-2715

25 **Stone NJ**, Bilek S, Rosenbaum S. Recent National Cholesterol Education Program Adult Treatment Panel III update: adjustments and options. *Am J Cardiol* 2005; **96**: 53E-59E

26 **World Health Organization Western Pacific Region, International Association for the Study of Obesity, International Obesity Task Force.** The Asia-Pacific Perspective: Redefining Obesity and Its Treatment. Sydney: Health Com-

munications, 2000
27 **Marchesini G**, Marzocchi R. Metabolic syndrome and NASH. *Clin Liver Dis* 2007; **11**: 105-117, ix
28 **Jimba S**, Nakagami T, Takahashi M, Wakamatsu T, Hirota Y, Iwamoto Y, Wasada T. Prevalence of non-alcoholic fatty liver disease and its association with impaired glucose metabolism in Japanese adults. *Diabet Med* 2005; **22**: 1141-1145
29 **Fan JG**, Zhu J, Li XJ, Chen L, Lu YS, Li L, Dai F, Li F, Chen SY. Fatty liver and the metabolic syndrome among Shanghai adults. *J Gastroenterol Hepatol* 2005; **20**: 1825-1832
30 **Amarapurkar DN**, Hashimoto E, Lesmana LA, Sollano JD, Chen PJ, Goh KL. How common is non-alcoholic fatty liver disease in the Asia-Pacific region and are there local differences? *J Gastroenterol Hepatol* 2007; **22**: 788-793
31 **Neuschwander-Tetri BA**, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. *Hepatology* 2003; **37**: 1202-1219
32 **Bedogni G**, Miglioli L, Masutti F, Tiribelli C, Marchesini G, Bellentani S. Prevalence of and risk factors for nonalcoholic fatty liver disease: the Dionysos nutrition and liver study. *Hepatology* 2005; **42**: 44-52
33 **Ford ES**, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *JAMA* 2002; **287**: 356-359
34 **Kotronen A**, Westerbacka J, Bergholm R, Pietiläinen KH, Yki-Järvinen H. Liver fat in the metabolic syndrome. *J Clin Endocrinol Metab* 2007; **92**: 3490-3497
35 **Sung KC**, Ryan MC, Wilson AM. The severity of nonalcoholic fatty liver disease is associated with increased cardiovascular risk in a large cohort of non-obese Asian subjects. *Atherosclerosis* 2009; **203**: 581-586
36 **Karnikowski M**, Córdova C, Oliveira RJ, Karnikowski MG, Nóbrega Ode T. Non-alcoholic fatty liver disease and metabolic syndrome in Brazilian middle-aged and older adults. *Sao Paulo Med J* 2007; **125**: 333-337

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BRIEF ARTICLE

Comparison of PPIs and H_2-receptor antagonists plus prokinetics for dysmotility-like dyspepsia

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Abstract

AIM: To compare efficacy of proton pump inhibitors (PPIs) with H_2-receptor antagonists (H_2RAs) plus prokinetics (Proks) for dysmotility-like symptoms in functional dyspepsia (FD).

METHODS: Subjects were randomized to receive open-label treatment with either rabeprazole 10 mg od (n = 57) or famotidine 10 mg bid plus mosapride 5 mg tid (n = 57) for 4 wk. The primary efficacy endpoint was change (%) from baseline in total dysmotility-like dyspepsia symptom score. The secondary efficacy endpoint was patient satisfaction with treatment.

RESULTS: The improvement in dysmotility-like dyspepsia symptom score on day 28 was significantly greater in the rabeprazole group (22.5% ± 29.2% of baseline) than the famotidine + mosapride group (53.2% ± 58.6% of baseline, $P < 0.0001$). The superior benefit of rabeprazole treatment after 28 d was consistent regardless of *Helicobacter pylori* status. Significantly more subjects in the rabeprazole group were satisfied or very satisfied with treatment on day 28 than in the famotidine + mosapride group (87.7% *vs* 59.6%, $P = 0.0012$). Rabeprazole therapy was the only significant predictor of treatment response ($P < 0.0001$), defined as a total symptom score improvement ⩾ 50%.

CONCLUSION: PPI monotherapy improves dysmotility-like symptoms significantly better than H_2RAs plus Proks, and should be the treatment of first choice for Japanese FD.

Key words: Dysmotility; Functional dyspepsia; H_2-receptor antagonist; Prokinetics; Proton pump inhibitor

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INTRODUCTION

Functional dyspepsia (FD) is a condition characterized by persistent upper abdominal symptoms in the absence of any causative organic disease[1]. It is thought to be caused by a combination of different factors, including dysmotility or hypersensitivity of the gastrointestinal (GI) tract, gastric acid secretion, inflammation of the gastric mucosa, altered sympathetic or parasympathetic activity, altered secretion of GI hormones, and psychological factors[2,3]. Treatments vary according to the symptoms, and include gastroprokinetic agents, suppressors of gastric acid secretion, antidepressants, anxiolytics and Chinese herbal medicines. Although it has been shown that gastric acid secretion is normal in patients with FD[4], a subset of these patients will benefit from strong acid suppression by a proton pump inhibitor (PPI)[5]. Inhibitors of acid secretion are therefore widely prescribed to patients with FD worldwide. Although treatment with acid suppression produces symptom relief in a proportion of patients with FD, this effect has not been reported consistently in all studies[6-8]. A Japanese study surveyed the prescribing habits of primary care physicians for functional GI symptoms and evaluated the efficacy and indications of the medications prescribed[9]. It was found that H_2-receptor antagonists (H_2RAs) are the treatment of first choice for ulcer-like symptoms such as epigastric pain, and prokinetics (Proks) for dysmotility-like symptoms such as epigastric discomfort, heaviness, and bloating. In other words, Japanese primary care physicians prefer H_2RA + prokinetic combination therapy for FD symptoms.

For FD patients with at least mild heartburn and/or regurgitation at baseline, omeprazole is associated with higher treatment success rates at 4 wk than ranitidine, cisapride and placebo[10]. In those patients who have either no or minimal heartburn and/or regurgitation at baseline, omeprazole and ranitidine are superior to placebo, although no significant difference is seen between omeprazole and ranitidine[10]. The question of whether more effective acid suppression is efficacious in Japanese patients with FD has yet to be adequately tested.

It is reasonable to think that H_2RAs provide adequate relief for FD symptoms in Japanese patients, who have a higher rate of *Helicobacter pylori* (*H. pylori*) infection than their western counterparts, as well as lower levels of gastric acid secretion[11].

Complete relief of symptoms is significantly more common with omeprazole than with placebo in subgroups of patients with ulcer-like and reflux-like dyspepsia, whereas, as might be expected, there is no indication of benefit with omeprazole in patients with dysmotility-like dyspepsia[5]. In addition, meta-analyses suggest that H_2RA and Proks are superior to placebo in non-ulcer dyspepsia (NUD)[12,13].

In this study, we concentrated on dysmotility-like symptoms in patients with FD, and compared the efficacy of PPI monotherapy and combination therapy with H_2RAs and Proks, which is widely prescribed by Japanese primary care physicians for FD symptoms.

MATERIALS AND METHODS

Selection of patients

This study was a randomized open-label trial conducted in three hospitals (Moriguchi Keijinkai Hospital, Osaka Saiseikai Nakatsu Hospital, and Arisawa General Hospital) and nine general medical clinics (Murotani Clinic, Majima Clinic, Morikawa Clinic, Hashimoto Clinic, Kiyota Clinic, Arisawa General Hospital, Amemoto Clinic, Isowa Clinic, and Mii Clinic) in Japan from January 2009 until April 2010.

The subjects were patients of at least 18 years of age with at least 1 mo of dyspepsia symptoms and no clinically significant findings at endoscopy. The main exclusion criteria were: (1) history of erosive esophagitis, peptic ulcer disease, GI malignancy, primary esophageal motility disorder, previous upper GI surgery; (2) maintenance treatment with a PPI or H_2RA within 2 wk of enrollment; and (3) severe concurrent disease. PPI, H_2RA and Prok use were not permitted during the 14 d prior to endoscopy, nor during the study. Nonsteroidal anti-inflammatory drugs, acetylsalicylic acid or steroids were not permitted at any time during the study. The study protocol was approved by the relevant Institutional Review Board and/or an Independent Ethics Committee, and informed written consent was obtained from each participating subject.

Study design

The investigators referred each enrolled subject for esophagogastroduodenoscopy. After endoscopy, eligible patients underwent a validated ^{13}C urea breath test to determine their *H. pylori* status. Subjects were randomly allocated to receive one of the following treatments for 4 wk: (1) rabeprazole 10 mg *od* (PPI); or (2) famotidine 10 mg *bid* plus mosapride 5 mg *tid* (H_2RA + Prok). Group allocations were assigned in equal numbers using a central computer-generated randomization list stratified for each participating institution. Subject compliance was assessed by counting the returned medication. Subjects were considered to have complied with treatment if they took at least 75% of the dispensed medication. Subjects attended their clinic at randomization and after 4 wk of treatment.

Symptom assessment

Subjects were asked to assess their dyspepsia symptoms at baseline and after 3 d, 7 d, 14 d and 28 d of treatment using a self-completed questionnaire for dyspepsia symp-

toms. Dysmotility-like dyspepsia symptoms were assessed using five questions (upper abdominal bloating, postprandial fullness, early satiation, belching, vomiting/nausea), and each response was graded on a five-point frequency scale as follows: 0, never; 1, occasionally; 2, sometimes; 3, often; 4, always. The scores for each question were totaled to give the total symptom score for dysmotility-like dyspepsia symptoms. The total symptom scores at each assessment time point were then expressed as a percentage of the baseline total symptom score.

Subject satisfaction

After 14 d and 28 d of treatment, subject satisfaction was evaluated using a four-grade scale as follows: very satisfied (symptoms disappeared); satisfied (symptoms improved considerably); somewhat satisfied (symptoms improved somewhat); unsatisfied (no improvement or symptoms worse).

Endpoints

The primary efficacy endpoint was the change (%) from baseline in total dysmotility-like dyspepsia symptom score. The secondary efficacy endpoint was subject satisfaction.

Sample size

The sample size calculation was based on the anticipated difference in symptom improvement rates between the PPI and H2RA + Prok groups. Due to the lack of clinical trials of H2RA + Prok combination therapy, we based our calculations of the sample size on the results of comparative trials of PPIs *vs* Proks.

The estimated success rate after 4 wk treatment was 23.7% for omeprazole, and 7.5% for cisapride[10]. Assuming a two-tailed α error rate of 0.05 and a power of 80%, with a 30% dropout rate during screening, 77.5 patients were required for each treatment arm.

Statistical analysis

Data are presented as mean ± SD. The intention-to-treat analysis included all randomized subjects. A subject who withdrew at any time was considered a dropout. We used the Wilcoxon single rank test for paired intra-individual comparisons, the Mann-Whitney *U* test for comparisons of continuous variables, and the χ^2 test for comparisons of categorized variables between the two treatment groups. In addition, we stratified primary endpoint results for differences between treatment groups according to *H. pylori* status. We performed multiple logistic regression analysis to determine factors (age, sex, *H. pylori* status, and baseline dysmotility-like dyspepsia symptom score) associated with treatment response (defined as change in total dysmotility-like dyspepsia symptom score of $\geqslant$ 50% after 28 d of treatment). $P < 0.05$ was considered to signify statistical significance for all analyses.

RESULTS

A total of 146 patients were randomized. Thirty-two patients were excluded in the follow-up period (30 lost to follow-up, two for non-compliance), leaving 114 patients for inclusion in the analysis. Fifty-seven patients were randomized to receive PPI treatment, and 57 to receive H2RA + Prok treatment (Figure 1). Baseline demographic characteristics and symptom scores of the patients who completed the treatment period are given in Table 1. There were no significant differences between the characteristics of the two treatment groups at baseline.

Table 1 Baseline demographic characteristics and total symptom scores for study completers

	H2RA + Prok (n = 57)	PPI (n = 57)	P value
Age (mean ± SD, yr)	51.5 ± 14.8	52.9 ± 13.8	0.6120
Sex (male/female)	17/40	12/45	0.3899
Helicobacter pylori infection (%)	43.8	43.8	> 0.9999
Symptom scores			
Upper abdominal bloating	1.6 ± 1.2	1.8 ± 1.3	0.6160
Postprandial fullness	2.1 ± 1.2	2.0 ± 1.2	0.7747
Early satiation	1.7 ± 1.3	1.3 ± 1.2	0.1934
Belching	1.7 ± 1.4	1.6 ± 1.4	0.7211
Vomiting/nausea	1.5 ± 1.3	1.4 ± 1.3	0.8361
Total symptom score (dysmotility-like dyspepsia symptoms)	8.6 ± 3.9	8.2 ± 4.7	0.5330

PPI: Proton pump inhibitor. H2RA: H2-receptor antagonist; Prok: Plus prokinetic.

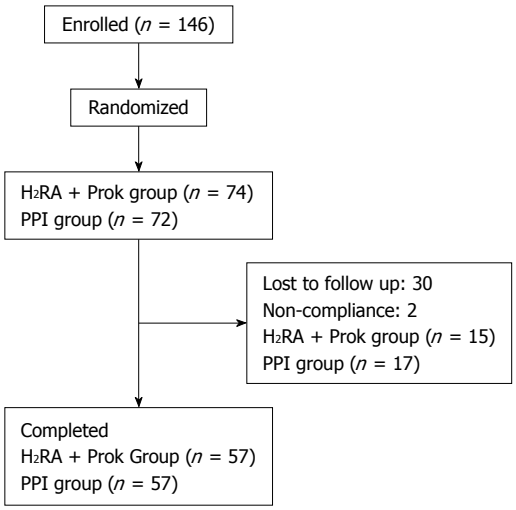

Figure 1 Study flow chart. This study enrolled 146 subjects with functional dyspepsia and, after excluding 30 subjects for non-attendance and two for non-compliance, 57 subjects in each treatment group completed the study.

Change in dysmotility-like dyspepsia symptom score

No significant differences were seen between groups in the change in dysmotility-like dyspepsia symptom score from baseline to 3 d or 7 d of treatment. After 28 d of treatment, the change in symptom score was significantly greater in the PPI group (22.5% ± 29.2% of baseline) than in the H2RA + Prok group (53.2% ± 58.6% of baseline) ($P < 0.0001$), indicating greater improvement in symptoms with PPI treatment (Figure 2). A significant improvement in total symptom score was seen over time in both groups, but in the H2RA + Prok group, the im-

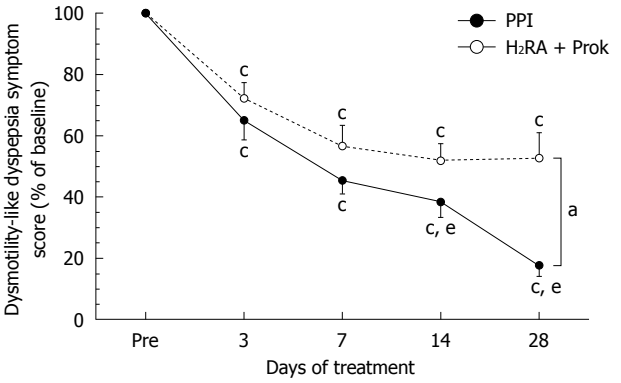

Figure 2 Change in dysmotility-like dyspepsia symptom score over time. Significantly greater improvement was seen in dysmotility-like dyspepsia symptom score on day 28 of treatment in the proton pump inhibitor (PPI) group than in the H_2-receptor antagonist (H_2RA) + prokinetic (Prok) group. No significant additional symptomatic improvement was seen after day 7 of H_2RA + Prok treatment, whereas further symptomatic improvements were seen over time in the PPI group. [a]$P < 0.05$, PPI *vs* H_2RA + Prok; [c]$P < 0.05$ *vs* pre-Rx in each group; [e]$P < 0.05$ *vs* 7 d Rx in each group.

provements in total symptom score on days 14 and 28 were not significantly greater than at day 7, whereas in the PPI group, the improvements in total symptom score at day 14 (38.4% ± 37.8% of baseline, $P = 0.0034$) and day 28 ($P < 0.0001$) were both significantly greater than on day 7 (45.1% ± 33.8% of baseline) (Figure 2).

Change in dysmotility-like dyspepsia symptom score according to H. pylori status

Among *H. pylori*-positive subjects, a significantly greater improvement in total symptom score was seen in the PPI group (13.4% ± 26.2% of baseline) than in the H_2RA + Prok group (53.4% ± 34.7% of baseline) ($P < 0.007$) by the end of the treatment period. Significant symptomatic improvement was seen over time in both treatment groups, although in the H_2RA + Prok group, no further improvement was observed after day 7 (35.7% ± 31.6% of baseline). In the PPI group, there was no significant difference between the changes in total symptom score for days 7 and 14, although there was a statistically significant difference between days 7 and 28 ($P = 0.0277$) (Figure 3A).

Among *H. pylori*-negative subjects, a significantly greater improvement in total symptom score was seen in the PPI group (24.1% ± 31.7% of baseline) than in the H_2RA + Prok group (53.1% ± 72.2% of baseline, $P < 0.0001$) on day 28. Significant symptomatic improvement was seen over time in both treatment groups, although the improvements seen in the H_2RA + Prok group on days 14 and 28 were not significantly superior to those observed on day 7. In the PPI group, the reductions in total symptom score on days 14 (44.2% ± 42.0% of baseline, $P = 0.0177$) and 28 ($P = 0.0002$) were both significantly greater than on day 7 (52.4% ± 34.1% of baseline) (Figure 3B).

Subject satisfaction on days 14 and 28 of treatment

Although no significant difference was seen between

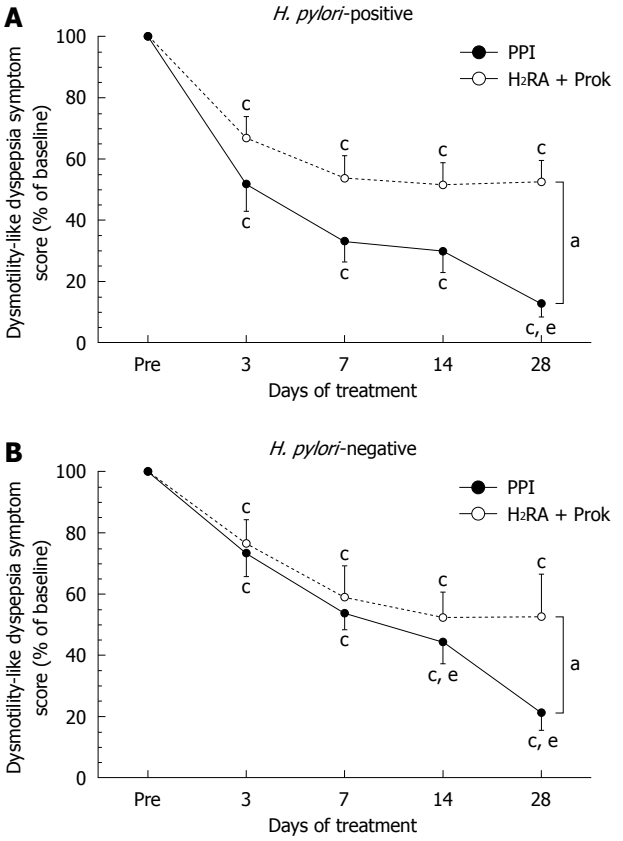

Figure 3 Change in dysmotility-like dyspepsia symptom score over time according to *Helicobacter pylori* status. Significantly greater symptomatic improvement was seen in the proton pump inhibitor group than in the H_2-receptor antagonist (H_2RA) + prokinetic (Prok) group after 28 d of treatment, regardless of *Helicobacter pylori* (*H. pylori*) status. No additional significant symptomatic improvement was seen after day 7 of H_2RA + Prok treatment, regardless of *H. pylori* status. [a]$P < 0.05$, PPI *vs* H_2RA + Prok; [c]$P < 0.05$ *vs* pre-Rx in each group; [e]$P < 0.05$ *vs* 7 d Rx in each group.

the groups on day 14, the proportion of subjects who were satisfied or very satisfied with their treatment was significantly higher in the PPI group than in the H_2RA + Prok group on day 28 (87.7% *vs* 59.6%, $P = 0.0012$). No significant increase was seen in subject satisfaction in the H_2RA + Prok group between days 14 and 28, whereas a significant increase was seen in the proportion of subjects in the PPI group answering satisfied or very satisfied between day 14 (63.2%) and 28 ($P = 0.0042$) (Figure 4).

Factors associated with treatment response

In this study, treatment response was defined as an improvement in dysmotility-like dyspepsia symptom score of ⩾ 50% after 28 d of treatment. The only factor identified by logistic regression analysis as a positive predictor of treatment response was PPI therapy (Table 2).

DISCUSSION

The Rome Ⅲ criteria define FD as "the chronic presence of one or more dyspepsia symptoms (bothersome postprandial fullness, early satiation, epigastric pain, epigastric burning) that are considered to originate from the

Table 2 Multiple logistic regression analysis of treatment response

Variable	Estimated OR	95% CI	*P* value
Age (yr)	0.999	0.962-1.036	0.9493
Sex: male	0.853	0.312-2.332	0.7563
Helicobacter pylori infection	0.521	0.191-1.419	0.2019
Treatment: PPI	9.055	3.231-25.376	< 0.0001
Dysmotility-like symptom score	1.062	0.940-1.200	0.3368

OR: Odds ratio; PPI: Proton pump inhibitor.

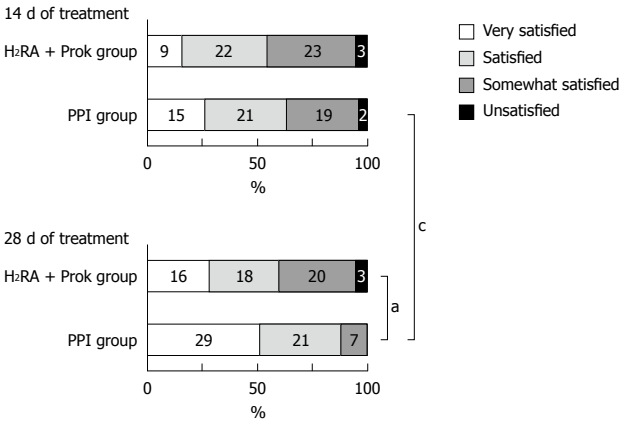

Figure 4 Subject satisfaction after 14 d and 28 d of treatment. Subject satisfaction was significantly higher in the proton pump inhibitor (PPI) group than in the H_2-receptor antagonist (H_2RA) + prokinetic (Prok) group on day 28 of treatment. No significant increase in subject satisfaction was seen in the H_2RA + Prok group between days 14 and 28, whereas a significant increase was seen in the PPI group between days 14 and 28. $^aP < 0.05$, PPI *vs* H_2RA + Prok; $^cP < 0.05$ *vs* 14 d Rx.

gastroduodenal region, with no evidence of structural disease (including at upper endoscopy) that is likely to explain the symptoms"[1]. FD is a common condition[14,15], with considerable adverse impact on quality of life[16], and represents a serious problem in everyday clinical practice. Patients with FD present with a variety of symptoms[17,18], so based on the symptom profile, they may be prescribed gastroprokinetic agents, suppressors of gastric acid secretion, antidepressants, anxiolytics or Chinese herbal medicines[19]. A Japanese survey of the prescribing habits of primary care physicians for upper GI symptoms has found that H_2RAs are prescribed for epigastric pain and heartburn, and Proks for epigastric discomfort, nausea and loss of appetite[9]. In other words, H_2RAs rather than PPIs are prescribed for epigastric pain syndrome[1], characterized by the two symptoms of epigastric pain and epigastric burning, unrelated to meals and considered mainly related to gastric acid. Proks are widely prescribed for postprandial distress syndrome[1], characterized by the two symptoms of postprandial fullness and early satiation, and considered to be strongly related to dysmotility of the GI tract; in particular, gastric accommodation of adaptive relaxation. H_2RA + Prok combination therapy is widely prescribed in Japan, where dyspepsia is not a recognized diagnosis for insurance purposes.

It has recently become clear that gastric acid secretion is strongly associated with the onset of dysmotility-like symptoms. Lee *et al*[20] have examined the influence of acid on gastric hypersensitivity and motility in healthy subjects, and have found that duodenal acidification significantly induces gastric hypersensitivity and impairs gastric motility. Compared with saline, infusion of acid into the duodenum causes not only ulcer reflux symptoms such as heartburn, but also dysmotility-like symptoms such as epigastric discomfort, belching and abdominal bloating[20]. When Miwa *et al*[21] infused acid into the stomach of Japanese subjects, they induced a variety of dyspeptic symptoms, with no significant change in acid-reflux-related symptoms such as heartburn and epigastric pain, but a significant increase in dysmotility-like symptoms such as epigastric discomfort, stomach fullness, nausea and belching. Samsom *et al*[22] have reported that decreased acid clearance and increased sensitivity to acid in patients with dyspepsia may lead to dyspeptic symptoms. There have in fact been a number of recent studies reporting the effectiveness of PPIs[7] and H_2RAs[23] in the treatment of FD. Empirical omeprazole therapy induces symptom improvement in a higher proportion of patients with uninvestigated dyspepsia (defined as epigastric pain or discomfort with or without heartburn or regurgitation) than ranitidine or cisapride does, but this effect is more marked in patients with gastroesophageal reflux disease[10]. The prevalence of pathological pH monitoring (4%-6% of time at pH < 4) is significantly higher in FD patients with heartburn than those without[17]. In other words, PPIs are more effective than H_2RAs in the treatment of FD associated with heartburn and regurgitation.

It is unclear, however, how important suppression of acid secretion is in Japanese patients, who have a high prevalence of *H. pylori* infection and low levels of gastric acid secretion[11]. A Japanese clinical trial found that H_2RA therapy was more effective than mosapride for treatment of FD[24], suggesting that suppression of gastric acid secretion by an H_2RA may be sufficient to treat FD in Japanese patients, with no need for a PPI. In this study, we compared PPI monotherapy with H_2RA + Prok combination therapy; the therapy most commonly prescribed by Japanese clinicians[9]. In consideration of the superior acid secretion suppression of PPIs over H_2RAs, we combined an H_2RA with a Prok, and examined only dysmotility-like symptoms, considered less responsive to PPI therapy.

Our results showed no differences between treatment groups for changes in dysmotility-like dyspepsia symptom score on days 3 and 7 of treatment, but significantly greater symptom improvement was seen in the PPI group than the H_2RA + Prok group on days 14 and 28 of treatment. The proportion of subjects reporting that they were satisfied or very satisfied was significantly higher in the PPI group than in the H_2RA + Prok group on day 28. PPI therapy was the only significant predictor

of treatment response, defined as a total symptom score improvement of ⩾ 50%.

In a recent meta-analysis of PPI treatment for FD, the dysmotility-like subgroup did not respond to PPI therapy, unlike the reflux and ulcer-like subgroup[25]. The severity of heartburn at baseline in FD patients influences treatment response to PPI or H2RA therapy[10]. We assessed heartburn symptoms at baseline in a separate study, finding no difference between groups in the pretreatment heartburn symptom score (data not shown). Our results suggest that powerful suppression of acid secretion by PPIs is also important in the treatment of dysmotility-like symptoms in Japanese patients with FD. We saw similar symptom score improvements in both groups until day 7 of treatment. This may represent a placebo response, because it is well known that the placebo response can be substantial in trials of GI disorders, including FD[10]. There have been reports of a placebo effect until day 7 of treatment with a PPI[26], and of the need for a 7-d run-in period to minimize the placebo effect in FD clinical trials[27-29], so a similar placebo response lasting until day 7 of treatment was also possible in this study. The absence of any further improvement in dysmotility-like dyspepsia symptoms between day 7 and days 14 or 28 in the H2RA + Prok group, and the lack of significant change in subject satisfaction rates from day 14 to 28, indicates the development of H2RA tolerance[30].

Stratifying the study sample by *H. pylori* status showed that significantly better symptom improvement was seen in the PPI group than the H2RA + Prok group in both *H. pylori*-positive and -negative subjects. No influence of *H. pylori* status was seen in either treatment group in our study. In the studies by Talley *et al*[5], no significant difference was seen in the rate of complete symptom resolution after 28 d of treatment between *H. pylori*-positive and -negative subjects in the ulcer-like, reflux-like, or dysmotility-like symptom subgroups in either treatment arm, although these were only exploratory analyses. Although *H. pylori* status did not significantly influence the response to omeprazole, this does not exclude the possibility of a small true difference between *H. pylori*-positive and -negative patients in the effect of acid inhibition on FD. Intragastric pH is higher in patients taking a PPI who are infected with *H. pylori*[31]. PPI therapy may therefore be more effective in *H. pylori*-positive patients with NUD, as one study has suggested[28]. Pooling all trial data suggests that symptom responses are similar in *H. pylori*-positive and -negative patients with NUD. *H. pylori* status is unlikely to have a clinically important impact on the efficacy of treatments in this patient group.

In this study, symptoms improved over time regardless of *H. pylori* status in the PPI group, whereas no further symptom improvement was seen in the H2RA + Prok group after day 7 of treatment in both *H. pylori*-positive and -negative subjects, indicating tolerance to treatment. Loss of the suppressive effect on acid secretion by H2RAs has been reported in *H. pylori*-negative patients as the duration of treatment increases[32], whereas in this study tolerance was seen from day 7 of treatment in the H2RA + Prok group.

Limitations of this study include the small number of subjects and the fact that it was not a placebo-controlled trial. Consideration of the Japanese medical system tells us that clinical trials of treatments for FD, a condition not covered by medical insurance, conducted with the participation of general medical clinics will be limited in scope. Nevertheless, this is the first published report of a randomized comparative trial of PPI monotherapy and H2RA + Prok combination therapy in the treatment of FD in Japanese subjects.

We demonstrated that, even in Japan with its high proportion of *H. pylori*-positive patients, PPI monotherapy significantly improves not only ulcer and reflux-like symptoms, but also dysmotility-like symptoms, better than H2RA + Prok combination therapy. In particular, tolerance to H2RA + Prok combination therapy was seen regardless of *H. pylori* status. The prevalence of *H. pylori* infection is expected to decline in Japan in the future[33], leading to increased gastric acid secretion, so suppression of acid secretion will likely become even more important. The American College of Gastroenterology guidelines for the management of dyspepsia recommend a PPI as the treatment of first choice in regions with a low prevalence of *H. pylori* infection, with investigations and other therapies for those who fail to respond[29]. In Japan, where the prevalence of *H. pylori* infection remains high, upper GI endoscopy is considered mandatory for the exclusion of malignancy. Powerful suppression of acid secretion, such as a PPI provides, is the most effective therapy for treatment of all dyspeptic symptoms in Japanese patients, both ulcer- and reflux-like and dysmotility-like symptoms. Of particular interest is our finding that PPI therapy is useful in the treatment of dysmotility-like symptoms, usually considered less responsive to PPIs. In other words, dysmotility-like symptoms of FD are also an acid-related disorder, for which suppression of acid secretion is the most effective therapy. PPIs are extremely effective in the treatment of all symptoms of FD, and should be the treatment of first choice in Japanese patients with FD.

COMMENTS

Background

Many people suffer from functional dyspepsia (FD). The disease has a substantial negative effect on quality of life, therefore, daily care is of the utmost importance. However, because there are many causes of FD, including gastrointestinal motility disorders and hypersensitivity of the digestive tract, gastric acid secretion, inflammation of the mucous membrane of the stomach, nervous system and digestive tract hormone disorders, and psychological factors, no method of treatment has been established.

Research frontiers

It has recently been clarified that gastric acid secretion plays a major role in the onset of FD symptoms. In Japan, however, where there are many *Helicobacter pylori* (*H. pylori*)-positive patients, epigastric pain, epigastric burning and other acid-related symptoms are treated with acid-secretion blockers. In contrast, dysmotility-like symptoms such as painful stomach heaviness after eating and soon feeling full are mainly treated with prokinetic agents and/or antiulcer agents, and the significance of acid secretion suppression is not clear. For this reason, we conducted a prospective randomized treatment study to examine

the importance of acid-suppressing drugs for the treatment of various FD symptoms.

Innovations and breakthroughs

Regardless of whether cases were *H. pylori*-positive or -negative, the improvement rate for dysmotility-like symptoms as well as ulcer and acid-reflux-related symptoms in a proton pump inhibitor (PPI) single therapy group was significantly better compared to a group concomitantly administered histamine H2 receptor antagonists (H_2RAs) and prokinetic drugs (Proks). Logistic analysis (multivariable analysis) of factors related to improvements in dysmotility-like symptoms also showed that PPI therapy was the only significant factor. Patient satisfaction was also significantly higher in the PPI single therapy group than in the H_2RA and Prok groups. In other words, in Japan, as well as other countries, regardless of whether a patient is *H. pylori*-positive or -negative, the importance of acid secretion suppression for the treatment of not only ulcer and acid-reflux-related symptoms but also digestive motility disorder symptoms has been established.

Applications

It is thought that the *H. pylori* infection rate in Japan will gradually decrease. Assuming this will lead to more patients with increased acid secretion, acid secretion suppression will become an increasingly important issue. It has been concluded that, regardless of whether a patient is *H. pylori*-positive or -negative, PPI therapy is extremely effective as a first-choice treatment for not only ulcer and acid-reflux-related symptoms but also dysmotility-like symptoms, in other words, for all FD symptoms.

Terminology

In the Rome Ⅲ classification, FD is defined as "one or more of the chronic symptoms of painful postprandial fullness, early feeling of satiety, epigastric pain and epigastric burning for which no causal organic disease is observed during endoscopic and other examinations". This definition is divided into the subcategories of epigastric pain syndrome, indicated by the two symptoms of epigastric pain related to dietary intake and epigastric burning related to gastric acid, and postprandial distress syndrome (PDS), indicated by the two so-called dysmotility-like symptoms of early satiation and fullness. Many cases of FD are thought to be due to PDS. In Japan, Proks, drugs that improve gastrointestinal motility, are the first-choice treatment for dysmotility-like symptoms. *H. pylori*, a bacterium that exists in the mucous membrane of the stomach, is thought to cause stomach and duodenal ulcers, and stomach cancer. In *H. pylori*-positive patients, the bacterium causes chronic atrophic gastritis, progressing to gastric cancer, and low gastric acid output. The *H. pylori*-positive rate in Japan is tending to decrease, which could result in more Japanese patients with excessive gastric acid output.

Peer review

A good paper and can be accepted for publication. Only problem is the number of patients recruited for the study was too low.

REFERENCES

1 **Tack J**, Talley NJ, Camilleri M, Holtmann G, Hu P, Malagelada JR, Stanghellini V. Functional gastroduodenal disorders. *Gastroenterology* 2006; **130**: 1466-1479
2 **Tack J**, Bisschops R, Sarnelli G. Pathophysiology and treatment of functional dyspepsia. *Gastroenterology* 2004; **127**: 1239-1255
3 **Choung RS**, Talley NJ. Novel mechanisms in functional dyspepsia. *World J Gastroenterol* 2006; **12**: 673-677
4 **Nyrén O**, Adami HO, Gustavsson S, Lindgren PG, Lööf L, Nyberg A. The "epigastric distress syndrome". A possible disease entity identified by history and endoscopy in patients with nonulcer dyspepsia. *J Clin Gastroenterol* 1987; **9**: 303-309
5 **Talley NJ**, Meineche-Schmidt V, Paré P, Duckworth M, Räisänen P, Pap A, Kordecki H, Schmid V. Efficacy of omeprazole in functional dyspepsia: double-blind, randomized, placebo-controlled trials (the Bond and Opera studies). *Aliment Pharmacol Ther* 1998; **12**: 1055-1065
6 **Wong WM**, Wong BC, Hung WK, Yee YK, Yip AW, Szeto ML, Fung FM, Tong TS, Lai KC, Hu WH, Yuen MF, Lam SK. Double blind, randomised, placebo controlled study of four weeks of lansoprazole for the treatment of functional dyspepsia in Chinese patients. *Gut* 2002; **51**: 502-506
7 **Peura DA**, Kovacs TO, Metz DC, Siepman N, Pilmer BL, Talley NJ. Lansoprazole in the treatment of functional dyspepsia: two double-blind, randomized, placebo-controlled trials. *Am J Med* 2004; **116**: 740-748
8 **Moayyedi P**, Soo S, Deeks J, Delaney B, Innes M, Forman D. Pharmacological interventions for non-ulcer dyspepsia. *Cochrane Database Syst Rev* 2004; CD001960
9 **Hongo M**, Kanatsuka H, Sugawara A, Nagasaki Y, Endo Y, Karahashi K, Shoji T, Sagami Y, Aoki I. Primary care in the treatment of functional gastrointestinal symptoms in Japan: prescription preferences and impression of results. *Aliment Pharmacol Ther* 2005; **21** Suppl 2: 47-54
10 **Veldhuyzen van Zanten SJ**, Chiba N, Armstrong D, Barkun A, Thomson A, Smyth S, Escobedo S, Lee J, Sinclair P. A randomized trial comparing omeprazole, ranitidine, cisapride, or placebo in helicobacter pylori negative, primary care patients with dyspepsia: the CADET-HN Study. *Am J Gastroenterol* 2005; **100**: 1477-1488
11 **Haruma K**, Kamada T, Kawaguchi H, Okamoto S, Yoshihara M, Sumii K, Inoue M, Kishimoto S, Kajiyama G, Miyoshi A. Effect of age and Helicobacter pylori infection on gastric acid secretion. *J Gastroenterol Hepatol* 2000; **15**: 277-283
12 **Allescher HD**, Böckenhoff A, Knapp G, Wienbeck M, Hartung J. Treatment of non-ulcer dyspepsia: a meta-analysis of placebo-controlled prospective studies. *Scand J Gastroenterol* 2001; **36**: 934-941
13 **Hiyama T**, Yoshihara M, Matsuo K, Kusunoki H, Kamada T, Ito M, Tanaka S, Nishi N, Chayama K, Haruma K. Meta-analysis of the effects of prokinetic agents in patients with functional dyspepsia. *J Gastroenterol Hepatol* 2007; **22**: 304-310
14 **Olafsdottir LB**, Gudjonsson H, Jonsdottir HH, Thjodleifsson B. Natural history of functional dyspepsia: a 10-year population-based study. *Digestion* 2010; **81**: 53-61
15 **Okumura T**, Tanno S, Ohhira M, Tanno S. Prevalence of functional dyspepsia in an outpatient clinic with primary care physicians in Japan. *J Gastroenterol* 2010; **45**: 187-194
16 **Jones RH**. Approaches to uninvestigated dyspepsia. *Gut* 2002; **50** Suppl 4: iv42-iv46
17 **Tack J**, Caenepeel P, Arts J, Lee KJ, Sifrim D, Janssens J. Prevalence of acid reflux in functional dyspepsia and its association with symptom profile. *Gut* 2005; **54**: 1370-1376
18 **Laheij RJ**, De Koning RW, Horrevorts AM, Rongen RJ, Rossum LG, Witteman EM, Hermsen JT, Jansen JB. Predominant symptom behavior in patients with persistent dyspepsia during treatment. *J Clin Gastroenterol* 2004; **38**: 490-495
19 **Halder SL**, Talley NJ. Functional Dyspepsia: A New Rome III Paradigm. *Curr Treat Options Gastroenterol* 2007; **10**: 259-272
20 **Lee KJ**, Vos R, Janssens J, Tack J. Influence of duodenal acidification on the sensorimotor function of the proximal stomach in humans. *Am J Physiol Gastrointest Liver Physiol* 2004; **286**: G278-G284
21 **Miwa H**, Nakajima K, Yamaguchi K, Fujimoto K, Veldhuyzen VAN Zanten SJ, Kinoshita Y, Adachi K, Kusunoki H, Haruma K. Generation of dyspeptic symptoms by direct acid infusion into the stomach of healthy Japanese subjects. *Aliment Pharmacol Ther* 2007; **26**: 257-264
22 **Samsom M**, Verhagen MA, vanBerge Henegouwen GP, Smout AJ. Abnormal clearance of exogenous acid and increased acid sensitivity of the proximal duodenum in dyspeptic patients. *Gastroenterology* 1999; **116**: 515-520
23 **Bytzer P**. H(2) receptor antagonists and prokinetics in dyspepsia: a critical review. *Gut* 2002; **50** Suppl 4: iv58-iv62
24 **Seno H**, Nakase H, Chiba T. Usefulness of famotidine in functional dyspepsia patient treatment: comparison among prokinetic, acid suppression and antianxiety therapies. *Aliment Pharmacol Ther* 2005; **21** Suppl 2: 32-36

25 **Moayyedi P**, Delaney BC, Vakil N, Forman D, Talley NJ. The efficacy of proton pump inhibitors in nonulcer dyspepsia: a systematic review and economic analysis. *Gastroenterology* 2004; **127**: 1329-1337
26 **van Rensburg C**, Berghöfer P, Enns R, Dattani ID, Maritz JF, Gonzalez Carro P, Fischer R, Schwan T. Efficacy and safety of pantoprazole 20 mg once daily treatment in patients with ulcer-like functional dyspepsia. *Curr Med Res Opin* 2008; **24**: 2009-2018
27 **Bolling-Sternevald E**, Lauritsen K, Aalykke C, Havelund T, Knudsen T, Unge P, Ekström P, Jaup B, Norrby A, Stubberöd A, Melén K, Carlsson R, Jerndal P, Junghard O, Glise H. Effect of profound acid suppression in functional dyspepsia: a double-blind, randomized, placebo-controlled trial. *Scand J Gastroenterol* 2002; **37**: 1395-1402
28 **Blum AL**, Arnold R, Stolte M, Fischer M, Koelz HR. Short course acid suppressive treatment for patients with functional dyspepsia: results depend on Helicobacter pylori status. The Frosch Study Group. *Gut* 2000; **47**: 473-480
29 **Talley NJ**, Vakil N, Lauritsen K, van Zanten SV, Flook N, Bolling-Sternevald E, Persson T, Björck E, Lind T. Randomized-controlled trial of esomeprazole in functional dyspepsia patients with epigastric pain or burning: does a 1-week trial of acid suppression predict symptom response? *Aliment Pharmacol Ther* 2007; **26**: 673-682
30 **Komazawa Y**, Adachi K, Mihara T, Ono M, Kawamura A, Fujishiro H, Kinoshita Y. Tolerance to famotidine and ranitidine treatment after 14 days of administration in healthy subjects without Helicobacter pylori infection. *J Gastroenterol Hepatol* 2003; **18**: 678-682
31 **Gillen D**, Wirz AA, Neithercut WD, Ardill JE, McColl KE. Helicobacter pylori infection potentiates the inhibition of gastric acid secretion by omeprazole. *Gut* 1999; **44**: 468-475
32 **Fujisawa T**, Adachi K, Komazawa Y, Mihara T, Azumi T, Katsube T, Furuta K, Kazumori H, Kinoshita Y. Helicobacter pylori infection prevents the occurrence of the tolerance phenomenon of histamine H2 receptor antagonists. *Aliment Pharmacol Ther* 2004; **20**: 559-565
33 **Fujisawa T**, Kumagai T, Akamatsu T, Kiyosawa K, Matsunaga Y. Changes in seroepidemiological pattern of Helicobacter pylori and hepatitis A virus over the last 20 years in Japan. *Am J Gastroenterol* 1999; **94**: 2094-2099

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BRIEF ARTICLE

Noninvasive Parameters and hepatic fibrosis scores in children with nonalcoholic fatty liver disease

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Abstract

AIM: To evaluate the noninvasive parameters and hepatic fibrosis scores in obese children with nonalcoholic fatty liver disease (NAFLD).

METHODS: A total of 77 children diagnosed with NAFLD *via* liver biopsy were included and divided into 2 subgroups according to the histopathologic staging of hepatic fibrosis: mild (stage 0-1) *vs* significant fibrosis (stage 2-4). Clinical and laboratory parameters were evaluated in each patient. The aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio, AST/platelet ratio index (APRI), PGA index, Forns index, FIB-4, NAFLD fibrosis score, and pediatric NAFLD fibrosis index (PNFI) were calculated.

RESULTS: No clinical or biochemical parameter exhibited a significant difference between patients with mild and significant fibrosis. Among noninvasive hepatic fibrosis scores, only APRI and FIB4 revealed a significant difference between patients with mild and significant fibrosis (APRI: 0.67 ± 0.54 *vs* 0.78 ± 0.38, $P = 0.032$ and FIB4: 0.24 ± 0.12 *vs* 0.31 ± 0.21, $P = 0.010$). The area under the receiving operating characteristic curve of FIB4 was 0.81, followed by Forns index (0.73), APRI (0.70), NAFLD fibrosis score (0.58), AST/ALT ratio (0.53), PGA score (0.45), and PNFI (0.41).

CONCLUSION: APRI and FIB4 might be useful noninvasive hepatic fibrosis scores for predicting hepatic fibrosis in children with NAFLD.

Key words: Fatty liver; Hepatic fibrosis; Noninvasive; Obesity; Child

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INTRODUCTION

The disease spectrum of nonalcoholic fatty liver disease (NAFLD) ranges from simple steatosis to nonalcoholic steatohepatitis (NASH), fibrosis, and cirrhosis; NASH progresses towards cirrhosis, even in children[1,2]. Therefore, accurate diagnosis and early detection of hepatic fibrosis are required in obese children suspicious of NAFLD.

Percutaneous liver biopsy is the gold standard for the diagnosis of NASH[3]. However, histopathologic investigation has some limitations because of its invasiveness and high costs, especially in children; moreover, the histopathologic findings of NAFLD in children are somewhat different from those in adults, revealing portal inflammation and portal fibrosis mainly in pediatric NASH in contrast to lobular inflammation, perisinusoidal fibrosis, ballooning, and Mallory's hyaline in adult NASH[4,5].

Noninvasive markers of hepatic fibrosis and noninvasive hepatic fibrosis scores have been evaluated in previous studies, mostly in adult patients with NAFLD[6]. Although pediatric NAFLD shows peculiar histopathologic features as described above[4,5], there have been only limited studies of noninvasive biochemical markers of hepatic fibrosis related to NAFLD in pediatric populations[7-10], and no validation studies in children on the previously suggested noninvasive hepatic fibrosis scores excluding 1 study on the enhanced liver fibrosis panel[11]. Only 1 pediatric noninvasive score has been developed up to date[12].

Therefore, the present study aimed to evaluate the noninvasive clinical and laboratory parameters and noninvasive hepatic fibrosis scores indicating the presence of hepatic fibrosis and its severity in obese children with NAFLD.

MATERIALS AND METHODS

A total of 77 obese children with NAFLD under the age of 18 years who visited the Pediatric Obesity Clinic were included and divided into 2 subgroups according to the grading and staging of NAFLD. Obesity was defined as a body mass index (BMI) value higher than the 95th percentile for the child's age and sex, and overweight as a BMI between the 85th and 95th percentiles. NAFLD was diagnosed on the basis of histopathologic findings compatible with NAFLD on liver biopsy[13]. This study was approved by the Institutional Review Board of the Seoul National University Bundang Hospital.

Laboratory tests

Serum levels of fasting glucose, insulin, total cholesterol, TGs, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and apoprotein A1 and B levels were measured after a 12-h fast at the same time of liver biopsy. Insulin resistance was determined by the homeostatic model assessment of insulin resistance. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, albumin, γ-glutamyl transpeptidase (γGT), alkaline phosphatase (ALP) levels, prothrombin time (PT), and tumor necrosis factor-α were also measured.

For the differential diagnosis of chronic hepatitis, creatine phosphokinase, lactate dehydrogenase, ammonia, lactate, pyruvate, anti-HAV IgM antibody, HBs antigen and anti-HBs antibody, anti-HCV antibody, EBV VCA IgM antibody, CMV IgM antibody, serum ceruloplasmin, and anti-nuclear antibody were evaluated.

Radiologic investigations

The presence of fatty liver was evaluated in each patient using either abdominal sonography or noncontrast abdominal computed tomography (CT). Using abdominal sonography, fatty liver was detected and the degree of fatty liver was defined as mild, moderate, and severe fatty liver[14]. Regarding abdominal CT, fatty liver was diagnosed when the difference between CT numbers of the liver and spleen was greater than 10[15].

Histopathologic examination

Percutaneous needle liver biopsy was performed in all patients. Histopathologic grades of steatosis, lobular and portal inflammation, and hepatocyte ballooning and the histopathologic stages of fibrosis were evaluated in each patient to diagnose NAFLD and to assess the stages of hepatic fibrosis[16]. The histological scoring system of Kleiner *et al*[13] was also applied to the liver biopsy specimens to assess the histologic stages of hepatic fibrosis and the NAFLD activity score (NAS). Fibrosis was staged as follows: stage 0: none; stage 1: perisinusoidal or periportal fibrosis (stage 1a: mild perisinusoidal; stage 1b: moderate perisinusoidal; stage 1c: portal/periportal; stage 2: perisinusoidal and portal/periportal fibrosis; stage 3: bridging fibrosis; stage 4: cirrhosis)[14].

Noninvasive hepatic fibrosis scores

The AST/ALT ratio was calculated as the ratio of AST to ALT[10]; AST/platelet ratio index (APRI) as follows: (AST level/AST upper level of normal/platelet counts) × 100[17]; PGA index as the sum of 3 scores based on the test results of PT, γGT activity, and apoprotein A1 and ranged from 0 to 12[18]; Forns index as follows: [7.811 - 3.131 × ln(platelet count) + 0.781 × ln(γGT) + 3.467 × ln(age) - 0.014 × cholesterol][19]; FIB-4 as (age × AST level/platelet count × √ALT)[20]; NAFLD fibrosis score as follows: [-1.675 + 0.037 × age (years) + 0.094 × BMI (kg/m^2) + 1.13 × impaired fasting glucose/diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio - 0.013 × platelet count - 0.66 × albumin][21]. Pediatric NAFLD fibrosis index (PNFI) was calculated using a formula suggested by Nobili *et al*[12].

Statistical analysis

The results are expressed as mean ± SD. The data were analyzed using the SPSS 18.0 software program (SPSS Inc., Chicago, IL, United States). Frequency data were compared using Fisher's exact test for nonparametric analysis. The Mann-Whitney *U* test was used for comparisons of means between 2 groups. Multivariate logistic regression analysis was performed to determine potential variables predicting significant hepatic fibrosis. *P*-values less than 0.05 were considered statistically significant.

Receiver operating characteristic (ROC) curves and the area under the ROC curve (AUROC) were applied to assess and compare the diagnostic accuracy of each noninvasive hepatic fibrosis scores.

RESULTS

Patient characteristics

A total of 77 children (M:F = 66:11; mean age 12.2 ±

Table 1 Patient characteristics and comparisons of noninvasive biochemical parameters between mild fibrosis and significant fibrosis regarding the hepatic histology of children with non-alcoholic fatty liver disease (mean ± SD)

Parameters	Total (n = 77)	Mild fibrosis (stage 0-1) (n = 51)	Significant fibrosis (stage 2-3) (n = 26)	P value
Clinical characteristics				
Gender (M:F)	66:11	42:9	24:2	0.316
Age (yr)	12.2 ± 2.3	12.0 ± 2.4	12.7 ± 1.9	0.132
AC (cm)	91.1 ± 10.1	92.1 ± 11.6	89.7 ± 7.8	0.637
Weight (kg)	68.9 ± 19.3	68.3 ± 21.3	70.2 ± 15.1	0.448
Height (cm)	155.5 ± 12.2	155.1 ± 12.1	156.3 ± 12.6	0.407
BMI (kg/m^2)	28.1 ± 5.1	27.8 ± 5.2	28.7 ± 5.1	0.425
Biochemical parameters				
AST (IU/L)	82.1 ± 46.6	77.4 ± 46.3	91.3 ± 46.8	0.063
ALT (IU/L)	167.4 ± 99.1	158.7 ± 95.4	184.6 ± 105.6	0.206
ALP (IU/L)	283.7 ± 110.0	273.0 ± 113.8	304.7 ± 101.1	0.185
γGT (IU/L)	53.9 ± 29.1	51.1 ± 25.9	59.4 ± 34.4	0.410
Total bilirubin (mg/dL)	0.76 ± 0.37	0.75 ± 0.30	0.77 ± 0.50	0.438
Albumin (g/dL)	4.54 ± 0.31	4.54 ± 0.32	4.55 ± 0.30	1.000
PT INR	1.02 ± 0.07	1.01 ± 0.06	1.03 ± 0.08	0.090
Total cholesterol (mg/dL)	180.2 ± 32.6	176.8 ± 31.2	186.9 ± 34.7	0.139
Triglyceride (mg/dL)	132.8 ± 53.6	126.6 ± 52.4	142.7 ± 55.0	0.235
LDL cholesterol (mg/dL)	97.2 ± 24.9	93.5 ± 20.6	103.1 ± 30.2	0.198
HDL cholesterol (mg/dL)	48.1 ± 11.4	48.9 ± 12.2	46.9 ± 10.2	0.995
Apoprotein A1 (mg/dL)	120.2 ± 15.9	121.6 ± 15.7	118.1 ± 16.8	0.543
Apoprotein B (mg/dL)	83.5 ± 17.7	85.6 ± 17.5	80.0 ± 18.3	0.418
Fasting glucose (mg/dL)	98.1 ± 34.2	95.4 ± 11.9	103.4 ± 56.7	0.311
Insulin (μIU/mL)	24.6 ± 14.6	23.2 ± 15.5	26.2 ± 13.5	0.244
HOMA-IR	5.8 ± 4.3	6.1 ± 4.8	5.3 ± 3.2	0.586
TNF-α (pg/mL)	18.2 ± 15.0	15.7 ± 12.3	21.7 ± 18.0	0.400
HbA1c (%)	5.7 ± 1.1	5.5 ± 0.3	5.9 ± 1.6	0.806
Platelet (× 10^9/L)	307.9 ± 69.2	313.2 ± 71.1	297.3 ± 65.4	0.416

AC: Abdominal circumference; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index; γGT: γ-glutamyl transpeptidase; DBP: Diastolic blood pressure; HDL: High density lipoprotein; HOMA-IR: Insulin resistance determined by homeostasis model assessment; LDL: Low density lipoprotein; NAFLD: Non-alcoholic fatty liver disease; NAS: NAFLD activity score; Ob: Obesity; Ow: Overweight; PT: Prothrombin time; SBP: Systolic blood pressure; TNF-α: Tumor necrosis factor-α.

2.2 years, range 8-18 years) diagnosed as NAFLD were included. The clinical characteristics of children with NAFLD are shown in Table 1.

Histopathologic findings and hepatic fibrosis in NAFLD

Histopathologic findings of the liver in children with NAFLD are listed in Table 2. Staging for hepatic fibrosis revealed the prevalence of stage 0 (n = 12), stage 1A (n = 12), stage 1B (n = 7), stage 1C (n = 20), stage 2 (n = 21), and stage 3 (n = 5). There was no patient with stage 4 of hepatic histology. Fibrosis stages were grouped into 2 subgroups: mild hepatic fibrosis (stage 0 to 1) (n = 51); and significant hepatic fibrosis (stage 2 to 3) (n = 26). The histopathologic findings for these 2 groups are compared in Table 2. There were no differences in steatosis, lobular inflammation, and NAS between 2 fibrosis groups (Table 2). Only hepatocyte ballooning was significantly different between the 2 groups (P = 0.018) (Table 2).

Noninvasive clinical and biochemical parameters in children

Clinical data of the patients and the results of laboratory tests for obesity and obesity-related complications are listed in Table 1. None of these noninvasive clinical and biochemical parameters exhibited a significant difference between the 2 fibrosis groups based on histopathologic findings (Table 1).

Multivariate logistic regression analysis for hepatic fibrosis in NAFLD

The result of multivariate logistic regression analysis for clinical or biochemical factors to predict significant hepatic fibrosis in children with NAFLD is shown in Table 3.

Comparisons of noninvasive hepatic fibrosis scores and hepatic fibrosis in children with NAFLD

The results of hepatic fibrosis scores including the AST/ALT ratio, APRI, PGA index, Forns index, FIB4, NAFLD fibrosis score, and PNFI were compared between the 2 groups based on the stage of hepatic fibrosis (Table 2).

Among the hepatic fibrosis scores, APRI and FIB4 revealed statistically significant differences between patients with mild fibrosis and significant fibrosis (APRI, P = 0.032; FIB4, P = 0.010) (Table 2). The other hepatic fibrosis scores were not significantly different between the 2 groups (AST/ALT ratio, P = 0.808; PGA index, P = 0.710; Forns index, P = 0.097; NAFLD fibrosis score, P = 0.532; PNFI, P = 0.314) (Table 2).

Table 2 Comparison of hepatic fibrosis scoring systems between mild fibrosis and significant fibrosis regarding the hepatic histology of children with non-alcoholic fatty liver disease (mean ± SD)

Parameters and hepatic fibrosis scores	Total (n = 77)	Mild fibrosis (stage 0-1) (n = 51)	Significant fibrosis (stage 2-3) (n = 26)	P value
Invasive histopathologic NAFLD scores[1]				
Steatosis (grade 0/1/2/3)	0/13/36/28	0/7/22/22	0/6/14/6	0.085
Inflammation (grade 0/1/2/3)	9/43/25/0	7/29/15/0	2/14/10/0	0.325
Ballooning (grade 0/1/2)	27/33/17	22/21/8	5/12/9	0.018
NAS	4.3 ± 1.4	4.18 ± 1.48	4.46 ± 1.14	0.470
Noninvasive hepatic fibrosis scoring systems				
AST/ALT ratio	0.53 ± 0.22	0.52 ± 0.16	0.57 ± 0.31	0.802
AST/platelet ratio index	0.71 ± 0.49	0.67 ± 0.54	0.78 ± 0.38	0.032
PGA index	3.78 ± 1.80	3.85 ± 1.89	3.68 ± 1.70	0.710
Forns index	-0.94 ± 1.18	-1.06 ± 0.21	-0.69 ± 1.09	0.097
FIB4 score	0.27 ± 0.16	0.24 ± 0.12	0.31 ± 0.21	0.010
NAFLD fibrosis score	-4.95 ± 1.32	-5.07 ± 1.27	-4.73 ± 1.41	0.532
Pediatric NAFLD fibrosis index	7.67 ± 2.48	7.71 ± 2.79	7.61 ± 2.08	0.314

[1]Histopathologic non-alcoholic fatty liver disease (NAFLD) scoring on liver biopsy specimens was based on the definition by Kleiner *et al*[13]. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; NAS: Non-alcoholic fatty liver disease activity score.

Table 3 Multivariate logistic regression analysis for significant hepatic fibrosis in children with non-alcoholic fatty liver disease

Variables	OR	95% CI	P value
Age	1.357	1.027-1.793	0.032
ALP	1.006	1.000-1.011	0.035

ALP: Alkaline phosphatase; OR: Odds ratio.

Comparison of the diagnostic accuracy of the hepatic fibrosis scoring systems

ROC curves of the hepatic fibrosis scoring systems are shown in Figure 1.

When the AUROC of each scoring system was compared, the AUROC of FIB4 was 0.81 (95% CI: 0.68-0.94), followed by the Forns index (AUROC = 0.73, 95% CI: 0.58-0.88), APRI (AUROC = 0.70, 95% CI: 0.55-0.86), NAFLD fibrosis score (AUROC = 0.58, 95% CI: 0.41-0.75), AST/ALT ratio (AUROC = 0.53, 95% CI: 0.35-0.70), PGA score (AUROC = 0.45, 95% CI: 0.28-0.62), and PNFI (AUROC = 0.41, 95% CI: 0.24-0.58).

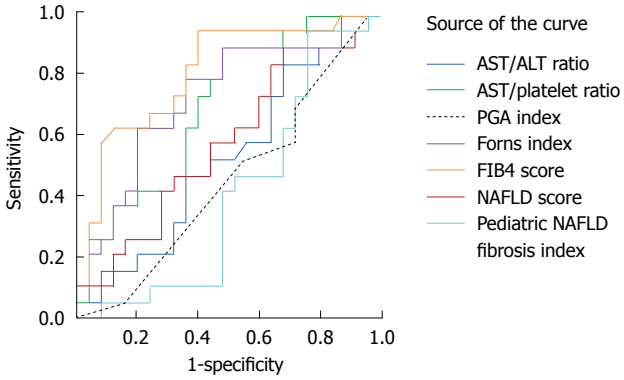

Figure 1 Receiver operating characteristic curves for the noninvasive hepatic fibrosis scoring systems used to diagnose clinically significant fibrosis (stage 2-3) in children with non-alcoholic fatty liver disease. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; NAFLD: Non-alcoholic fatty liver disease.

DISCUSSION

Due to the invasiveness of histopathologic diagnosis based on liver biopsy, noninvasive methods including the measurement of various clinical parameters or laboratory markers have been applied to clinical practice and research, mostly in adults, to facilitate the diagnosis of NAFLD and to predict hepatic fibrosis in obese patients.

In our study performed in children with biopsy-proven NAFLD, both clinical and biochemical parameters were evaluated on the basis of the histopathologic findings of liver biopsy specimens, particularly focused on biochemical parameters. However, all of these clinical and biochemical parameters failed to distinguish significant fibrosis from no/mild fibrosis in children with NAFLD by univariate analysis, although the result of multivariate analysis suggested age and ALP as possible prognostic factors that predict significant hepatic fibrosis.

Regarding the clinical markers in children with NAFLD, 1 multicenter study reached the same conclusion as our study, revealing no significantly different clinical parameters for significant fibrosis (stage 2 or more), and the serum AST level was the only biochemical parameter associated with the severity of hepatic fibrosis[9]. In contrast, another pediatric study reported that BMI was the only clinical parameter that significantly differentiated NAFLD with hepatic fibrosis from NAFLD without fibrosis, and there were no biochemical parameters predictive of hepatic fibrosis[10]. From these results, it appears that more developed noninvasive tools beyond simple parameters are needed to detect hepatic fibrosis in children suspicious of NAFLD.

To this point, a number of noninvasive hepatic fibrosis scores have been developed and applied to chronic liver diseases, such as hepatitis B, hepatitis C, alcoholic fatty

liver disease, and NAFLD[22,23]. These scores include indices based on indirect biochemical markers and/or clinical parameters developed for adults, such as the AST/ALT ratio[10], APRI[17], PGA index[18], Forns index[19], FIB-4[20], and NAFLD fibrosis score[21]. These scores have been validated in a number of previously published studies in adults[22], but no validation studies have been performed in children with NAFLD, excluding the AST/ALT ratio, which did not differentiate hepatic fibrosis from no fibrosis in children[10].

In our study, only APRI and FIB4 exhibited statistically significant differences between patients with mild fibrosis and those with significant fibrosis among these noninvasive scores. Regarding APRI, no studies have been reported in children with NAFLD. However, a study on APRI in children with chronic viral hepatitis revealed that the AUROC of APRI was 0.71 for hepatic fibrosis, and the sensitivity and the specificity of APRI were 47% and 90%, respectively, at the cutoff of 0.5[24]. In addition, another study comparing APRI with FibroScan and FibroTest in children with chronic liver disease reported the AUROC of APRI to be 0.73[25]. In our study on children with NAFLD, the AUROC of APRI was 0.70, which was similar to those of previous studies on children with chronic liver diseases.

FIB4, comprising age in addition to AST, ALT, and platelet counts, also differentiated significant hepatic fibrosis from mild fibrosis in our study, with the highest AUROC of 0.81. In previous studies on FIB4 in adult patients with NAFLD, the AUROCs of FIB4 were 0.802 and 0.86, respectively, which were higher than those of the AST/ALT ratio, APRI, NAFLD fibrosis score, and the BMI, AST/ALT ratio, diabetes score in both studies[26,27]. FIB4 was significant in distinguishing advanced fibrosis (stage 3 to 4) from mild fibrosis (stage 0 to 2) in these 2 studies[26,27]. These results were similar to those of our study, indicating that FIB4 had the highest AUROC among a variety of hepatic fibrosis scores using standard laboratory tests. Thus, FIB4 also appears to be a desirable noninvasive hepatic fibrosis score, even in children, with the application of age-appropriate cutoffs.

Up to date, PNFI was the only noninvasive hepatic fibrosis scoring system developed for children with NAFLD, which revealed the AUROC for significant fibrosis (stage 2 to 3) was 0.663[28], and according to previous studies, PNFI was significant in differentiating children with hepatic fibrosis from children without fibrosis[12,28]. However, in our study, PNFI failed to distinguish significant hepatic fibrosis from no/mild fibrosis in children with the lowest AUROC of 0.41.

Because histopathologic findings of pediatric NASH are to some extent distinct from adult NASH[4,5], the application of noninvasive hepatic fibrosis scores to children with NAFLD should be considered from a different point of view. More validation studies may be required in the future to apply noninvasive hepatic fibrosis scoring systems to clinical fields in pediatric population.

In conclusion, it appears that no single clinical or laboratory parameter can reflect the presence of hepatic fibrosis or the severity of fibrosis in children with NAFLD. Therefore, APRI and FIB4 might be regarded as useful noninvasive methods to evaluate hepatic fibrosis even in children with NAFLD.

COMMENTS

Background

Although liver biopsy is the gold standard for the diagnosis of nonalcoholic fatty liver disease (NAFLD), it has some limitations in children because of its invasiveness and high costs, and moreover, the histopathologic findings of pediatric nonalcoholic steatohepatitis (NASH) are somewhat different from adult NASH. Thus, noninvasive methods to predict hepatic fibrosis are required in children suspicious of NAFLD.

Research frontiers

The application of noninvasive hepatic fibrosis scores to detect liver fibrosis may be useful in detecting and predicting significant hepatic fibrosis in pediatric NAFLD.

Innovations and breakthroughs

There have been only limited studies of noninvasive markers or hepatic fibrosis scores of hepatic fibrosis related to NAFLD in pediatric populations. This is the first study applying noninvasive clinical or biochemical markers and various noninvasive hepatic fibrosis scores together to the detection of hepatic fibrosis. Our study suggests that AST/platelet ratio index and FIB4, among hepatic fibrosis scores, might be useful to detect hepatic fibrosis even in children with NAFLD.

Applications

The study may represent a future diagnostics for NAFLD in pediatric population, suggesting APRI and FIB4 as noninvasive tools to evaluate hepatic fibrosis in children possibly instead of invasive liver biopsy.

Terminology

NAFLD is a form of chronic liver disease with histopathologic features of alcoholinduced liver disease occurs in persons who do not consume a significant amount of alcohol. Because the main etiology of NAFLD is obesity, the prevalence of NAFLD is increasing in children as the prevalence of pediatric obesity is increasing.

Peer review

In this study, authors investigate several non-invasive scores for liver fibrosis in children with established and histology-proven NAFLD. This study is of interest, as today only view reports about the diagnostic accuracy of these scores in children are available.

REFERENCES

1 **Powell EE**, Cooksley WG, Hanson R, Searle J, Halliday JW, Powell LW. The natural history of nonalcoholic steatohepatitis: a follow-up study of forty-two patients for up to 21 years. *Hepatology* 1990; **11**: 74-80

2 **Molleston JP**, White F, Teckman J, Fitzgerald JF. Obese children with steatohepatitis can develop cirrhosis in childhood. *Am J Gastroenterol* 2002; **97**: 2460-2462

3 **Straub BK**, Schirmacher P. Pathology and biopsy assessment of non-alcoholic fatty liver disease. *Dig Dis* 2010; **28**: 197-202

4 **Schwimmer JB**, Behling C, Newbury R, Deutsch R, Nievergelt C, Schork NJ, Lavine JE. Histopathology of pediatric nonalcoholic fatty liver disease. *Hepatology* 2005; **42**: 641-649

5 **Ko JS**, Yoon JM, Yang HR, Myung JK, Kim H, Kang GH, Cheon JE, Seo JK. Clinical and histological features of nonalcoholic fatty liver disease in children. *Dig Dis Sci* 2009; **54**: 2225-2230

6 **Guha IN**, Parkes J, Roderick PR, Harris S, Rosenberg WM. Non-invasive markers associated with liver fibrosis in nonalcoholic fatty liver disease. *Gut* 2006; **55**: 1650-1660

7 **Nobili V**, Alkhouri N, Alisi A, Ottino S, Lopez R, Manco M, Feldstein AE. Retinol-binding protein 4: a promising circulating marker of liver damage in pediatric nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2009; **7**: 575-579

8 **Nobili V**, Alisi A, Torre G, De Vito R, Pietrobattista A, Morino G, De Ville De Goyet J, Bedogni G, Pinzani M. Hyaluronic acid predicts hepatic fibrosis in children with nonalcoholic fatty liver disease. *Transl Res* 2010; **156**: 229-234

9 **Carter-Kent C**, Yerian LM, Brunt EM, Angulo P, Kohli R, Ling SC, Xanthakos SA, Whitington PF, Charatcharoenwitthaya P, Yap J, Lopez R, McCullough AJ, Feldstein AE. Nonalcoholic steatohepatitis in children: a multicenter clinicopathological study. *Hepatology* 2009; **50**: 1113-1120

10 **Iacobellis A**, Marcellini M, Andriulli A, Perri F, Leandro G, Devito R, Nobili V. Non invasive evaluation of liver fibrosis in paediatric patients with nonalcoholic steatohepatitis. *World J Gastroenterol* 2006; **12**: 7821-7825

11 **Nobili V**, Parkes J, Bottazzo G, Marcellini M, Cross R, Newman D, Vizzutti F, Pinzani M, Rosenberg WM. Performance of ELF serum markers in predicting fibrosis stage in pediatric non-alcoholic fatty liver disease. *Gastroenterology* 2009; **136**: 160-167

12 **Nobili V**, Alisi A, Vania A, Tiribelli C, Pietrobattista A, Bedogni G. The pediatric NAFLD fibrosis index: a predictor of liver fibrosis in children with non-alcoholic fatty liver disease. *BMC Med* 2009; **7**: 21

13 **Kleiner DE**, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41**: 1313-1321

14 **Strauss S**, Gavish E, Gottlieb P, Katsnelson L. Interobserver and intraobserver variability in the sonographic assessment of fatty liver. *AJR Am J Roentgenol* 2007; **189**: W320-W323

15 **Ricci C**, Longo R, Gioulis E, Bosco M, Pollesello P, Masutti F, Crocè LS, Paoletti S, de Bernard B, Tiribelli C, Dalla Palma L. Noninvasive in vivo quantitative assessment of fat content in human liver. *J Hepatol* 1997; **27**: 108-113

16 **Brunt EM**, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999; **94**: 2467-2474

17 **Loaeza-del-Castillo A**, Paz-Pineda F, Oviedo-Cárdenas E, Sánchez-Avila F, Vargas-Vorácková F. AST to platelet ratio index (APRI) for the noninvasive evaluation of liver fibrosis. *Ann Hepatol* 2008; **7**: 350-357

18 **Teare JP**, Sherman D, Greenfield SM, Simpson J, Bray G, Catterall AP, Murray-Lyon IM, Peters TJ, Williams R, Thompson RP. Comparison of serum procollagen III peptide concentrations and PGA index for assessment of hepatic fibrosis. *Lancet* 1993; **342**: 895-898

19 **Forns X**, Ampurdanès S, Llovet JM, Aponte J, Quintó L, Martínez-Bauer E, Bruguera M, Sánchez-Tapias JM, Rodés J. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. *Hepatology* 2002; **36**: 986-992

20 **Vallet-Pichard A**, Mallet V, Nalpas B, Verkarre V, Nalpas A, Dhalluin-Venier V, Fontaine H, Pol S. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. comparison with liver biopsy and fibrotest. *Hepatology* 2007; **46**: 32-36

21 **Angulo P**, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, Enders F, Saksena S, Burt AD, Bida JP, Lindor K, Sanderson SO, Lenzi M, Adams LA, Kench J, Therneau TM, Day CP. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology* 2007; **45**: 846-854

22 **Poynard T**, Morra R, Ingiliz P, Imbert-Bismut F, Thabut D, Messous D, Munteanu M, Massard J, Benhamou Y, Ratziu V. Biomarkers of liver fibrosis. *Adv Clin Chem* 2008; **46**: 131-160

23 **Adams LA**. Biomarkers of liver fibrosis. *J Gastroenterol Hepatol* 2011; **26**: 802-809

24 **McGoogan KE**, Smith PB, Choi SS, Berman W, Jhaveri R. Performance of the AST-to-platelet ratio index as a noninvasive marker of fibrosis in pediatric patients with chronic viral hepatitis. *J Pediatr Gastroenterol Nutr* 2010; **50**: 344-346

25 **de Lédinghen V**, Le Bail B, Rebouissoux L, Fournier C, Foucher J, Miette V, Castéra L, Sandrin L, Merrouche W, Lavrand F, Lamireau T. Liver stiffness measurement in children using FibroScan: feasibility study and comparison with Fibrotest, aspartate transaminase to platelets ratio index, and liver biopsy. *J Pediatr Gastroenterol Nutr* 2007; **45**: 443-450

26 **Shah AG**, Lydecker A, Murray K, Tetri BN, Contos MJ, Sanyal AJ. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2009; **7**: 1104-1112

27 **McPherson S**, Stewart SF, Henderson E, Burt AD, Day CP. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. *Gut* 2010; **59**: 1265-1269

28 **Alkhouri N**, Carter-Kent C, Lopez R, Rosenberg WM, Pinzani M, Bedogni G, Feldstein AE, Nobili V. A combination of the pediatric NAFLD fibrosis index and enhanced liver fibrosis test identifies children with fibrosis. *Clin Gastroenterol Hepatol* 2011; **9**: 150-155

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BRIEF ARTICLE

Clinical implication of 14-3-3 epsilon expression in gastric cancer

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Abstract

AIM: To evaluate for the first time the protein and mRNA expression of 14-3-3ε in gastric carcinogenesis.

METHODS: 14-3-3ε protein expression was determined by western blotting, and mRNA expression was examined by real-time quantitative RT-PCR in gastric tumors and their matched non-neoplastic gastric tissue samples.

RESULTS: Authors observed a significant reduction of 14-3-3ε protein expression in gastric cancer (GC) samples compared to their matched non-neoplastic tissue. Reduced levels of 14-3-3ε were also associated with diffuse-type GC and early-onset of this pathology. Our data suggest that reduced 14-3-3ε may have a role in gastric carcinogenesis process.

CONCLUSION: Our results reveal that the reduced 14-3-3ε expression in GC and investigation of 14-3-3ε interaction partners may help to elucidate the carcinogenesis process.

Key words: Gastric cancer; 14-3-3 epsilon; YWHAE; Gene expression; Protein expression

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INTRODUCTION

Although gastric cancer (GC) rates have decreased substantially in most parts of the world, it is still the fourth most frequent cancer type and the second highest cause of cancer mortality worldwide. A total of 989 600 new

stomach cancer cases and 738 000 deaths are estimated to have occurred in 2008, accounting for 8% of the total cases and 10% of total deaths by cancer[1].

About 90% of stomach tumors are adenocarcinomas[2]. However, the etiology and disease evolution may vary among populations, primary tumor location, histological subtypes of adenocarcinoma, and other variables[3].

GC, as with other neoplasms, is a multifactorial disease that results from a combination of environmental factors and accumulation of generalized and specific genetic and epigenetic alterations. Chromosomal instability is characterized by changes in chromosome copy number (aneuploidy) and alterations in chromosomal regions, which may induce oncogene activation, tumor suppressor gene inactivation, or both[4]. The chromosomal aberrations that are constantly found in GC include gains of 3q, 7p, 7q, 8q, 13q, 17q, 20p and 20q and losses of 4q, 9p, 17p and 18q (for a review, see[5]). Our research group previously reported that the loss of one copy of *TP53* locus (17p13) is commonly found in gastric tumors of individuals from a Brazilian population[6], as well as in GC cell lines[7-9]. Although *TP53* is a key tumor suppressor gene in the carcinogenesis process[10], additional genes at 17p13 may play a role in gastric carcinogenesis.

The *YWHAE* gene is located at 17p13.3 and encodes the 14-3-3ε protein, one of the mammalian 14-3-3 protein family members that are highly conserved in eukaryotes. There are at least seven distinct *14-3-3* genes in vertebrates, giving rise to nine isoforms (α, β, γ, δ, ε, ζ, η, σ and τ/υ, with α and δ being phosphorylated forms of β and ζ, respectively)[11,12]. The 14-3-3 proteins are predominantly dimeric within the cell and bind either to multiple sites within single proteins or act as a bridge between two targets[13-15]. Up to now, > 300 proteins have been reported to interact with 14-3-3 proteins, including key signaling components, such as p53, Raf-1 kinase, Bcl-2 antagonist of cell death, protein kinase C, phophatidylinositol 3-kinase, and cdc25 phosphatase (RASGRF1)[12,13,15,16]. Although the exact 14-3-3 protein functions are not fully known, these proteins may act as a molecular scaffold, bringing together proteins that interact functionally and effecting phosphorylation-dependent cell regulation[12]. This protein family is involved in several biological processes and plays a regulatory role in processes such as apoptotic cell death, mitogenic signal transduction, and cell cycle control[13,17,18].

The isoform 14-3-3ε is the most highly conserved member of the 14-3-3 family, with conserved sequence in plants, yeast, and mammals[19,20]. Abnormal expression of 14-3-3ε has been found in some types of cancers. However, the role of 14-3-3ε in the carcinogenesis process is ambiguous and contradictory. Low expression of 14-3-3ε occurs in small cell lung cancer[21], laryngeal squamous cell carcinoma[22], and medulloblastoma[23], which suggest its role as a tumor suppressor gene. On the other hand, high expression of 14-3-3ε has been detected in renal carcinoma[24], astrocytoma[25], meningioma[26] and subependymomas[27], and, thus, probably it acts as a oncogene.

To the best of our knowledge, no study has evaluated the role of 14-3-3ε in gastric carcinogenesis until now. In the present study, we analyzed the *14-3-3ε* gene and protein expression in GC and matched non-neoplastic gastric samples. We also evaluated the possible associations between 14-3-3ε and clinicopathological characteristics.

MATERIALS AND METHODS

Tissue samples

14-3-3ε protein expression was evaluated in 20 pairs of GC samples and corresponding non-neoplastic gastric tissues (distant location of primary tumor). The mRNA expression was evaluated in 31 pairs of GC samples and corresponding non-neoplastic gastric tissues. Dissected tumor and paired non-neoplastic tissue specimens were immediately cut from stomach samples, frozen in liquid nitrogen, and stored at -80 ℃ until use for protein and RNA extraction. All the gastric samples were obtained surgically from João de Barros Barreto University Hospital (HUJBB) in Pará State, Northern Brazil. Informed consent with approval of the ethics committee of HUJBB was obtained. All patients had negative histories of exposure to either chemotherapy or radiotherapy before surgery and there was no other co-occurrence of diagnosed cancers. All samples were classified according to Laurén[28] and tumors were staged using standard criteria by TNM staging[29].

Protein and mRNA purification

Total protein and total mRNA were simultaneously isolated from gastric tissue samples using the AllPrep DNA/RNA/Protein Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. To allow greatest solubilization, the protein pellet was dissolved in buffer containing 7 mol urea, 2 mol thiourea, 4% CHAPS, 50 mmol dithiothreitol, 1% Protease Inhibitor Cocktail (Sigma, St Louis, MO, United States), and 0.5% of each Phosphatase Inhibitor Cocktail 1 and 2 (Sigma-Aldrich, St Louis, MO, United States). Protein concentration was determined by the method of Bradford (Sigma-Aldrich). RNA concentration and quality were determined using a NanoDrop spectrophotometer (Kisker, Germany) and 1% agarose gels. Samples were stored at -80 ℃ until use.

14-3-3ε expression by western blotting

Reduced protein (30 μg) of each sample was separated on 12.5% homogeneous SDS-PAGE gel and electroblotted to a polyvinylidene fluoride (PVDF) membrane (Hybond-*P*; GE Healthcare, Uppsala, Sweden). The PVDF membrane was blocked with PBS containing 0.1% Tween 20, 5% low-fat milk, and incubated overnight at 4 ℃ with corresponding primary antibodies to anti-14-3-3ε (sc-31962, 1:100; Santa Cruz Biotechnology, Santa Cruz, CA, United States) and anti-β-actin (Ac-74, 1:3000; Sigma-Aldrich). After extensive washing, a peroxidase-conjugated secondary antibody was incubated for 1 h at room temperature. Immunoreactive bands were visualized using western blotting Luminol reagent and the images were acquired using an ImageQuant 350 digital image system (GE Healthcare, Uppsala, Sweden). The β-actin was used as a loading reference control.

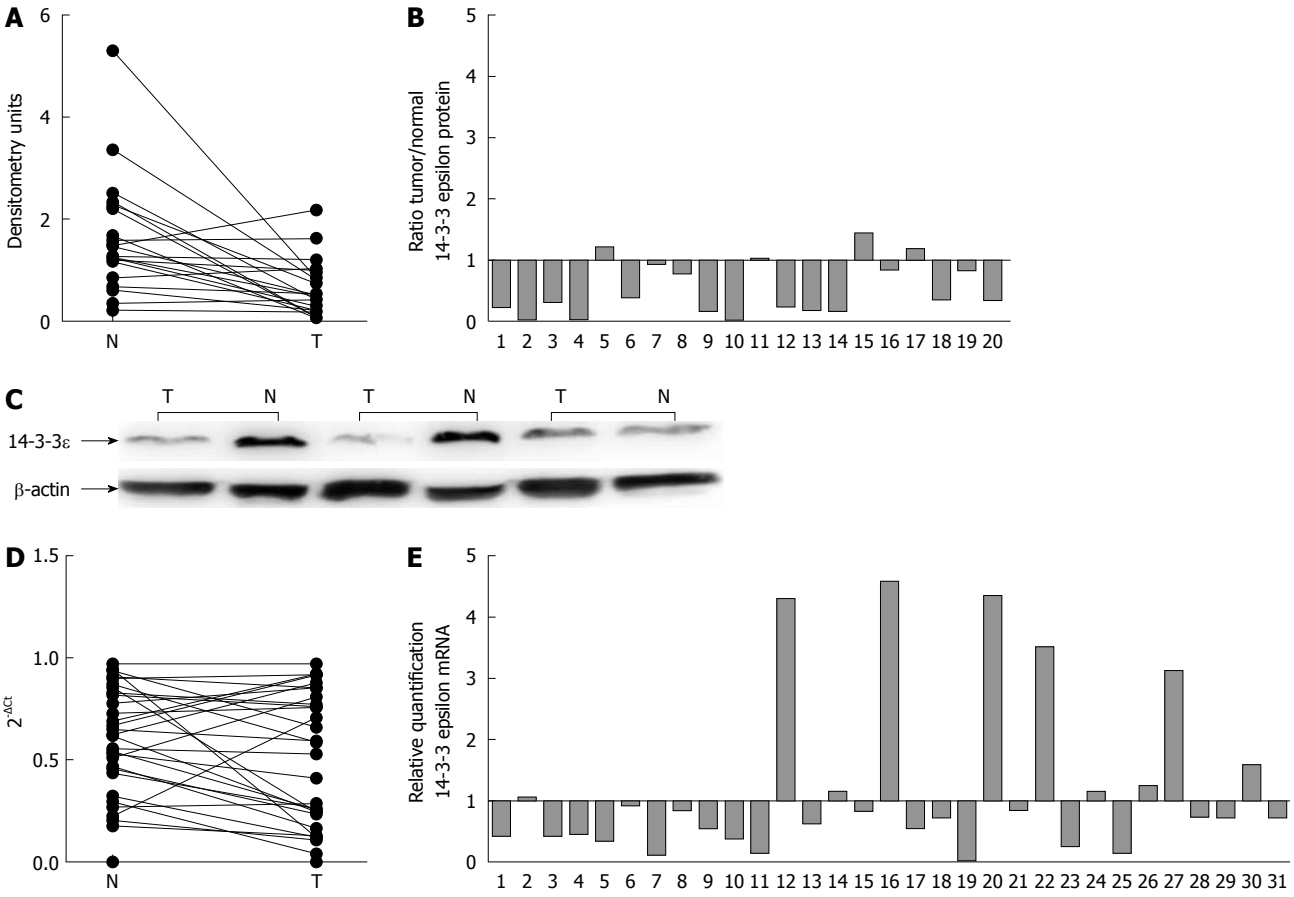

Figure 1 Expression of 14-3-3ε in tumor and non-neoplastic gastric tissue. A: Protein expression normalized by β-actin (ACTB); B: Ratio of protein expression between tumor and matched non-neoplastic gastric tissues; C: Western blotting using anti-14-3-3ε and anti-β-actin antibodies; D: mRNA expression normalized by the internal controls ACTB and glyceraldehyde-3-phosphate dehydrogenase; E: Relative mRNA quantification-gastric tumor samples normalized by matched non-neoplastic gastric tissues. T: Tumor samples; N: Non-neoplastic samples.

14-3-3ε mRNA expression by real-time quantitative RT-PCR

First, cDNA was synthesized using High-Capacity cDNA Archive kit (Applied Biosystems, Warsaw, Poland) according to the manufacturer's protocol. All real-time qRT-PCR reactions were performed in triplicate for both target gene (YWHAE: Hs00356749_g1, Applied Biosystems, United States) and internal controls (β-actin: Hs03023943_g1; glyceraldehyde-3-phosphate dehydrogenase: Hs99999905_m1; Applied Biosystems, United States). To compare 14-3-3ε mRNA expression between GC and non-neoplastic gastric samples, we converted ΔCt (ΔCt = Ct of *YWHAE* - Ct of internal controls) to linear form ($2^{-\Delta Ct}$). Relative quantification of the gene expression was calculated according to Pfaffl method[30]. Non-neoplastic gastric samples were designated as a calibrator of each paired tumor sample.

Statistical analysis

We first evaluated the normal distribution of all data using the Shapiro-Wilk normality test to determine subsequent use of appropriate tests for statistical comparison. Since 14-3-3ε mRNA and protein data did not present with a normal distribution, we performed parametric tests with bootstrapping, a re-sampling method. The re-sampling methods are relatively powerful and can control a type I error (false positive), reducing over-fit bias and internally validating the accuracy estimates. Bootstrapping methods also produce confidence intervals (CIs) around the observed effects. Paired *t* test was performed to compare the mean of 14-3-3ε expression between neoplastic and matched non-neoplastic samples. The associations between clinicopathological parameters and the mean of 14-3-3ε expression were assessed using a *t* test for independent samples. The correlation among the 14-3-3ε mRNA and protein expression was analyzed by Pearson test. All the analyses performed in this article were based on 1000 bootstrap samples. In all analyses, the CI was 95% and $P < 0.05$ was considered significant.

RESULTS

14-3-3ε protein expression was significantly reduced in GC samples (densitometry units: 0.656 ± 0.552) compared to matched non-neoplastic gastric samples (1.656 ± 1.158) ($P = 0.005$, 95% CI: -1.59 to -0.53). 14-3-3ε protein was 1.5-fold lower in 60% of GC samples compared to their paired non-neoplastic gastric tissues. However, 14-3-3ε mRNA expression did not differ between GC ($2^{-\Delta Ct}$: 0.492 ± 0.325) and corresponding non-neoplastic gastric tissue (0.579 ± 0.272) ($P = 0.075$, 95% CI: -0.18 to 0.003) (Figure 1). Although mRNA and protein were simultaneously purified,

Table 1 Clinicopathological characteristics and 14-3-3ε expression in gastric cancer samples (mean ± SD)

Variable	14-3-3ε protein expression				14-3-3ε mRNA expression			
	Total	Ratio T/N	Adjusted *P*-value	95% CI	Total	Relative quantification	Adjusted *P*-value	95% CI
Gender								
Male	10	0.418 ± 0.41	0.210	-0.13 to 0.63	18	1.404 ± 1.43	0.279	-1.38 to 0.40
Female	10	0.667 ± 0.47			13	0.898 ± 1.09		
Onset (yr)								
< 45	6	0.303 ± 0.26	0.052	-0.68 to -0.05	8	0.583 ± 0.31	0.028[a]	-1.49 to -0.25
⩾ 45	14	0.646 ± 0.48			23	1.403 ± 1.45		
Tumor location								
Cardia	3	0.293 ± 0.11	0.067	0.03 to 0.56	4	0.796 ± 0.35	0.177	-0.12 to 1.23
Non-cardia	17	0.587 ± 0.47			27	1.250 ± 1.39		
Laurén classification								
Diffuse-type	4	0.244 ± 0.14	0.024[a]	0.12 to 0.65	13	1.119 ± 1.04	0.785	-0.69 to 1.07
Intestinal-type	16	0.618 ± 0.46			18	1.245 ± 1.49		
Stage								
Early	4	0.517 ± 0.64	0.921	-0.55 to 0.81	4	0.549 ± 0.38	0.046[a]	-1.41 to -0.16
Advanced	16	0.549 ± 0.41			27	1.287 ± 1.37		
Tumor invasion								
T1/T2	8	0.491 ± 0.49	0.674	-0.48 to 0.34	9	0.642 ± 0.33	0.052	-1.40 to -0.13
T3/T4	12	0.578 ± 0.43			22	1.417 ± 1.49		
Lymph node metastasis								
Absent	6	0.672 ± 0.57	0.510	-0.29 to 0.72	8	0.687 ± 0.53	0.069	-1.31 to -0.01
Present	14	0.488 ± 0.39			23	1.367 ± 1.45		
Distant metastasis								
Unknown/absent	16	0.557 ± 0.47	0.754	-0.42 to 0.47	23	0.978 ± 0.99	0.242	-2.25 to 0.51
Present	4	0.486 ± 0.37			8	1.807 ± 1.89		

Differentially expressed between groups, [a]$P < 0.05$. T: Tumor gastric samples; N: Non-neoplastic gastric samples.

no correlation was observed between 14-3-3ε mRNA and protein expression ($R = 0.236$, $P = 0.345$).

The associations between clinicopathological characteristics and 14-3-3ε expression are summarized in Table 1. A significant decrease in 14-3-3ε mRNA level was observed in early-onset GC (at age ⩽ 45 years[31]) compared to late-onset GC ($P = 0.028$, 95% CI: -1.49 to -0.25). Moreover, a tendency towards 14-3-3ε protein down-expression was also observed in early-onset compared to late-onset GC ($P = 0.052$, 95% CI: -0.69 to -0.05).

The 14-3-3ε mRNA level was reduced in early GC compared to advanced GC ($P = 0.046$, 95% CI: -1.41 to -0.16) and tended to present reduced levels with less invasive tumors ($P = 0.052$, 95% CI: -1.40 to -0.13). In contrast, these observations were not observed for protein level.

Concerning the 14-3-3ε protein, we observed that the diffuse-type GC presented reduced expression compared to intestinal-type GC ($P = 0.024$, 95% CI: 0.12-0.65). However, a significant difference was observed between non-neoplastic gastric tissues and intestinal-type GC ($P = 0.045$, 95% CI: -1.55 to -0.29) and diffuse-type GC ($P = 0.024$, 95% CI: -2.22 to -1.14).

DISCUSSION

Cancer cells have defects in regulatory circuits that govern normal cell proliferation and homeostasis[32]. The 14-3-3 proteins continue to generate intense interest due to their roles in signal transduction pathways that control cell cycle checkpoints, mitogen-activate protein kinase activation, apoptosis, and regulation of gene expression. 14-3-3 stabilizes non-native conformations of bound ligands to promote their interactions with downstream targets, or facilitates their subsequent modification by kinases and phosphatases[18].

In the present study, we evaluated the 14-3-3ε mRNA and protein expression in gastric carcinogenesis. To the best of our knowledge, no study has evaluated 14-3-3ε expression in this neoplasm. Here, we observed that 14-3-3ε protein expression was reduced in GC samples compared to matched non-neoplastic gastric tissue. Low expression of 14-3-3ε has also been reported in small cell lung cancer[21], laryngeal squamous cell carcinoma[22], and medulloblastoma[23], suggesting a tumor suppressor function. The reduced 14-3-3ε expression may be in part due to the loss of its locus (17p13), which is a common finding in gastric tumors of individuals from the studied population[6].

Little is known about the role of 14-3-3ε in carcinogenesis. One of the major partners of 14-3-3ε is the CDC25 protein. CDC25 protein is virtually inactive during interphase, but undergoes a strong activation at mitosis due to phosphorylation of its N-terminal regulatory domain[33]. 14-3-3ε acts as a negative regulator of CDC25[33,34]. The overexpression of CDC25 proteins has previously been described in GC[35-37]. Thus, the reduced expression of 14-3-3ε in GC cells may enhance the ability of CDC25 to induce mitosis, contributing to the gastric carcinogenesis process.

Several classification systems have been described for

GC. According to the Laurén classification, one of the most used, gastric adenocarcinoma is classified mainly into intestinal and diffuse types[28]. Intestinal-type GC progresses through a number of sequential steps, beginning with atrophic gastritis followed by intestinal metaplasia, intraepithelial neoplasia, and carcinoma[38]. In contrast, diffuse-type GC generally does not evolve from precancerous lesions[39,40]. In the present study, we observed that the expression of 14-3-3ε protein was lower in diffuse-type than in intestinal-type GC, confirming that these two histological GC subtypes follow different genetic pathways and may be two distinct entities[39].

MYC deregulation is a frequent finding in GC[41]. Moreover, MYC immunoreactivity seems to be more frequently detected in intestinal-type than diffuse-type GC[42,43]. Gene amplification is the main mechanism of MYC deregulation in GC[41], and we have previously described a higher frequency of *MYC* locus amplification in intestinal-type than diffuse-type GC[44,45]. Interestingly, one of the MYC target proteins is CDC25, which is negatively regulated by 14-3-3ε. A correlation between MYC and CDC25 has previously been reported in GC[46]. Here, we hypothesize that the relative increase of 14-3-3ε in the subset of tumors of the intestinal-type may be a compensatory mechanism to control the increase of cell proliferation due to MYC and CDC25 action. Moreover, MYC overexpression also induces the production of reactive oxygen species, which may lead to double-stranded DNA breaks and point mutations resulting in genomic instability[47]. Thus, the relative increase in 14-3-3ε in the intestinal-type GC may be also a compensatory response to other oncogenic mutations accumulated in cancer cells, which lead to genomic instability and initiate a DNA damage check-point in cells that contain some functional p53 alleles, as already proposed for other 14-3-3 isoforms[18].

Here, we also described a decrease in 14-3-3ε expression in early-onset compared to late-onset GC. Early-onset GC is observed in < 10% of GC patients, and only 10% of these patients have a positive family history[31]. Most young patients present at an advanced clinical stage similar to elderly patients, so the prognosis in both age groups is poor. However, early-onset GC shows different clinicopathological and molecular profiles compared to late-onset GC, suggesting that they represent a separate entity within gastric carcinogenesis, with genetic factors probably presenting a more important role in early-onset GC patients[48-50]. 14-3-3ε deregulation may have a direct or indirect function in early-onset GC because this protein interacts with several others.

In addition, no correlation was observed between 14-3-3ε mRNA and protein expression, corroborating a previous study of laryngeal squamous cell carcinoma[22]. The lack of correlation between 14-3-3ε protein and mRNA expression patterns indicates the post-translational regulation mechanism involved in this protein expression, and highlights the complexity of the relationship between protein and mRNA expression.

In conclusion, our data suggest that, for the first time, reduced 14-3-3ε may have a role in gastric carcinogenesis, mainly in diffuse-type and early-onset GC. Moreover, further investigations are necessary to understand which proteins interact with 14-3-3ε in these subtypes of GC.

COMMENTS

Background

Gastric cancer (GC) is the fourth most frequent cancer type and the second highest cause of cancer mortality worldwide. Although this neoplasm is a serious public health problem due to its high incidence and mortality, little is known about the molecular events involved in gastric carcinogenesis.

Research frontiers

GC, as with other neoplasms, is a multifactorial disease that results from a combination of environmental factors and the accumulation of generalized and specific genetic and epigenetic alterations. The 14-3-3 protein family has been recently associated with carcinogenesis, but not in the stomach. In this study, the authors evaluated mRNA and protein expression of 14-3-3ε in gastric neoplasms and corresponding non-neoplastic samples.

Innovations and breakthroughs

No previous study has evaluated the gene and protein expression of 14-3-3ε in gastric carcinogenesis. This study demonstrates that 14-3-3ε has a role in gastric carcinogenesis, especially in diffuse-type and early-onset GC.

Applications

14-3-3ε may have a role in gastric carcinogenesis as a tumor suppressor protein.

Terminology

The *YWHAE* gene is located at 17p13.3, a region frequently deleted in gastric neoplasms. This gene encodes the 14-3-3ε protein, one of the mammalian 14-3-3 protein family members that are highly conserved in eukaryotes. This protein family is involved in several biological processes and plays a regulatory role in processes such as apoptotic cell death, mitogenic signal transduction, and cell cycle control.

Peer review

The results report for the first time the expression of 14-3-3ε in GC, both at the protein and mRNA level, and the data obtained reveal reduced 14-3-3ε protein expression in GC. The data contribute to the biology of GC with possible future clinical implications to be tested in a subsequent study in a larger number of GC patients.

REFERENCES

1 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90
2 **Crew KD**, Neugut AI. Epidemiology of gastric cancer. *World J Gastroenterol* 2006; **12**: 354-362
3 **Shah MA**, Ajani JA. Gastric cancer--an enigmatic and heterogeneous disease. *JAMA* 2010; **303**: 1753-1754
4 **Ottini L**, Falchetti M, Lupi R, Rizzolo P, Agnese V, Colucci G, Bazan V, Russo A. Patterns of genomic instability in gastric cancer: clinical implications and perspectives. *Ann Oncol* 2006; **17** Suppl 7: vii97-vi102
5 **Panani AD**. Cytogenetic and molecular aspects of gastric cancer: clinical implications. *Cancer Lett* 2008; **266**: 99-115
6 **Khayat AS**, Guimarães AC, Calcagno DQ, Seabra AD, Lima EM, Leal MF, Faria MH, Rabenhorst SH, Assumpção PP, Demachki S, Smith MA, Burbano RR. Interrelationship between TP53 gene deletion, protein expression and chromosome 17 aneusomy in gastric adenocarcinoma. *BMC Gastroenterol* 2009; **9**: 55
7 **Leal MF**, Martins do Nascimento JL, da Silva CE, Vita Lamarão MF, Calcagno DQ, Khayat AS, Assumpção PP, Cabral IR, de Arruda Cardoso Smith M, Burbano RR. Establishment and conventional cytogenetic characterization of three gastric cancer cell lines. *Cancer Genet Cytogenet* 2009; **195**: 85-91

8 **Ribeiro HF**, Alcântara DF, Matos LA, Sousa JM, Leal MF, Smith MA, Burbano RR, Bahia MO. Cytogenetic characterization and evaluation of c-MYC gene amplification in PG100, a new Brazilian gastric cancer cell line. *Braz J Med Biol Res* 2010; **43**: 717-721

9 **Leal MF**, Calcagno DQ, Borges da Costa Jde F, Silva TC, Khayat AS, Chen ES, Assumpção PP, de Arruda Cardoso Smith M, Burbano RR. MYC, TP53, and chromosome 17 copy-number alterations in multiple gastric cancer cell lines and in their parental primary tumors. *J Biomed Biotechnol* 2011; **2011**: 631268

10 **Szymańska K**, Hainaut P. TP53 and mutations in human cancer. *Acta Biochim Pol* 2003; **50**: 231-238

11 **Aitken A**. 14-3-3 proteins: a historic overview. *Semin Cancer Biol* 2006; **16**: 162-172

12 **Fu H**, Subramanian RR, Masters SC. 14-3-3 proteins: structure, function, and regulation. *Annu Rev Pharmacol Toxicol* 2000; **40**: 617-647

13 **van Hemert MJ**, Steensma HY, van Heusden GP. 14-3-3 proteins: key regulators of cell division, signalling and apoptosis. *Bioessays* 2001; **23**: 936-946

14 **Pozuelo Rubio M**, Geraghty KM, Wong BH, Wood NT, Campbell DG, Morrice N, Mackintosh C. 14-3-3-affinity purification of over 200 human phosphoproteins reveals new links to regulation of cellular metabolism, proliferation and trafficking. *Biochem J* 2004; **379**: 395-408

15 **Satoh J**, Nanri Y, Yamamura T. Rapid identification of 14-3-3-binding proteins by protein microarray analysis. *J Neurosci Methods* 2006; **152**: 278-288

16 **Rajagopalan S**, Sade RS, Townsley FM, Fersht AR. Mechanistic differences in the transcriptional activation of p53 by 14-3-3 isoforms. *Nucleic Acids Res* 2010; **38**: 893-906

17 **Tzivion G**, Avruch J. 14-3-3 proteins: active cofactors in cellular regulation by serine/threonine phosphorylation. *J Biol Chem* 2002; **277**: 3061-3064

18 **Yaffe MB**. How do 14-3-3 proteins work?-- Gatekeeper phosphorylation and the molecular anvil hypothesis. *FEBS Lett* 2002; **513**: 53-57

19 **Aitken A**. Functional specificity in 14-3-3 isoform interactions through dimer formation and phosphorylation. Chromosome location of mammalian isoforms and variants. *Plant Mol Biol* 2002; **50**: 993-1010

20 **Jones DH**, Ley S, Aitken A. Isoforms of 14-3-3 protein can form homo- and heterodimers in vivo and in vitro: implications for function as adapter proteins. *FEBS Lett* 1995; **368**: 55-58

21 **Konishi H**, Nakagawa T, Harano T, Mizuno K, Saito H, Masuda A, Matsuda H, Osada H, Takahashi T. Identification of frequent G(2) checkpoint impairment and a homozygous deletion of 14-3-3epsilon at 17p13.3 in small cell lung cancers. *Cancer Res* 2002; **62**: 271-276

22 **Che XH**, Chen H, Xu ZM, Shang C, Sun KL, Fu WN. 14-3-3epsilon contributes to tumour suppression in laryngeal carcinoma by affecting apoptosis and invasion. *BMC Cancer* 2010; **10**: 306

23 **Cvekl A**, Zavadil J, Birshtein BK, Grotzer MA, Cvekl A. Analysis of transcripts from 17p13.3 in medulloblastoma suggests ROX/MNT as a potential tumour suppressor gene. *Eur J Cancer* 2004; **40**: 2525-2532

24 **Liang S**, Xu Y, Shen G, Liu Q, Zhao X, Xu Z, Xie X, Gong F, Li R, Wei Y. Quantitative protein expression profiling of 14-3-3 isoforms in human renal carcinoma shows 14-3-3 epsilon is involved in limitedly increasing renal cell proliferation. *Electrophoresis* 2009; **30**: 4152-4162

25 **Cao L**, Cao W, Zhang W, Lin H, Yang X, Zhen H, Cheng J, Dong W, Huo J, Zhang X. Identification of 14-3-3 protein isoforms in human astrocytoma by immunohistochemistry. *Neurosci Lett* 2008; **432**: 94-99

26 **Liu Y**, Tian RF, Li YM, Liu WP, Cao L, Yang XL, Cao WD, Zhang X. The expression of seven 14-3-3 isoforms in human meningioma. *Brain Res* 2010; **1336**: 98-102

27 **Lukashova-v Zangen I**, Kneitz S, Monoranu CM, Rutkowski S, Hinkes B, Vince GH, Huang B, Roggendorf W. Ependymoma gene expression profiles associated with histological subtype, proliferation, and patient survival. *Acta Neuropathol* 2007; **113**: 325-337

28 **Lauren P**. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. an attempt at a histo-clinical classification. *Acta Pathol Microbiol Scand* 1965; **64**: 31-49

29 **Sobin LH**, Wittekind CH. TNM classification of malignant tumours. 6th ed. New York: Wiley-Liss, 2002

30 **Pfaffl MW**. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 2001; **29**: e45

31 **Kokkola A**, Sipponen P. Gastric carcinoma in young adults. *Hepatogastroenterology* 2001; **48**: 1552-1555

32 **Hanahan D**, Weinberg RA. The hallmarks of cancer. *Cell* 2000; **100**: 57-70

33 **Kumagai A**, Yakowec PS, Dunphy WG. 14-3-3 proteins act as negative regulators of the mitotic inducer Cdc25 in Xenopus egg extracts. *Mol Biol Cell* 1998; **9**: 345-354

34 **Kumagai A**, Dunphy WG. Binding of 14-3-3 proteins and nuclear export control the intracellular localization of the mitotic inducer Cdc25. *Genes Dev* 1999; **13**: 1067-1072

35 **Kudo Y**, Yasui W, Ue T, Yamamoto S, Yokozaki H, Nikai H, Tahara E. Overexpression of cyclin-dependent kinase-activating CDC25B phosphatase in human gastric carcinomas. *Jpn J Cancer Res* 1997; **88**: 947-952

36 **Zhang XM**, Yao GY, Zhang BY, Wang LL, Zhao M. [Study on the expression and significance of Galectin-3 and CDC25B mRNA in human gastric carcinoma]. *Zhonghua Yixue Yichuanxue Zazhi* 2009; **26**: 288-292

37 **Takahashi H**, Murai Y, Tsuneyama K, Nomoto K, Okada E, Fujita H, Takano Y. High labeling indices of cdc25B is linked to progression of gastric cancers and associated with a poor prognosis. *Appl Immunohistochem Mol Morphol* 2007; **15**: 267-272

38 **Correa P**. Human gastric carcinogenesis: a multistep and multifactorial process--First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. *Cancer Res* 1992; **52**: 6735-6740

39 **Tahara E**. Genetic pathways of two types of gastric cancer. *IARC Sci Publ* 2004; 327-349

40 **Smith MG**, Hold GL, Tahara E, El-Omar EM. Cellular and molecular aspects of gastric cancer. *World J Gastroenterol* 2006; **12**: 2979-2990

41 **Calcagno DQ**, Leal MF, Assumpcao PP, Smith MA, Burbano RR. MYC and gastric adenocarcinoma carcinogenesis. *World J Gastroenterol* 2008; **14**: 5962-5968

42 **Kozma L**, Kiss I, Hajdú J, Szentkereszty Z, Szakáll S, Ember I. C-myc amplification and cluster analysis in human gastric carcinoma. *Anticancer Res* 2001; **21**: 707-710

43 **Yang GF**, Deng CS, Xiong YY, Gong LL, Wang BC, Luo J. Expression of nuclear factor-kappa B and target genes in gastric precancerous lesions and adenocarcinoma: association with Helicobactor pylori cagA (+) infection. *World J Gastroenterol* 2004; **10**: 491-496

44 **Burbano RR**, Assumpção PP, Leal MF, Calcagno DQ, Guimarães AC, Khayat AS, Takeno SS, Chen ES, De Arruda Cardoso Smith M. C-MYC locus amplification as metastasis predictor in intestinal-type gastric adenocarcinomas: CGH study in Brazil. *Anticancer Res* 2006; **26**: 2909-2914

45 **Calcagno DQ**, Leal MF, Taken SS, Assumpção PP, Demachki S, Smith Mde A, Burbano RR. Aneuploidy of chromosome 8 and C-MYC amplification in individuals from northern Brazil with gastric adenocarcinoma. *Anticancer Res* 2005; **25**: 4069-4074

46 **Xing X**, Chen J, Chen M. Expression of CDC25 phosphatases in human gastric cancer. *Dig Dis Sci* 2008; **53**: 949-953
47 **Prochownik EV**, Li Y. The ever expanding role for c-Myc in promoting genomic instability. *Cell Cycle* 2007; **6**: 1024-1029
48 **Carvalho R**, Milne AN, van Rees BP, Caspers E, Cirnes L, Figueiredo C, Offerhaus GJ, Weterman MA. Early-onset gastric carcinomas display molecular characteristics distinct from gastric carcinomas occurring at a later age. *J Pathol* 2004; **204**: 75-83
49 **Milne AN**, Carvalho R, Morsink FM, Musler AR, de Leng WW, Ristimäki A, Offerhaus GJ. Early-onset gastric cancers have a different molecular expression profile than conventional gastric cancers. *Mod Pathol* 2006; **19**: 564-572
50 **Milne AN**, Sitarz R, Carvalho R, Carneiro F, Offerhaus GJ. Early onset gastric cancer: on the road to unraveling gastric carcinogenesis. *Curr Mol Med* 2007; **7**: 15-28

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CASE REPORT

Pancreatic schwannoma: Case report and an updated 30-year review of the literature yielding 47 cases

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Abstract

Pancreatic schwannomas are rare neoplasms. Authors briefly describe a 64-year-old female patient with cystic pancreatic schwannoma mimicking other cystic tumors and review the literature. Databases for PubMed were searched for English-language articles from 1980 to 2010 using a list of keywords, as well as references from review articles. Only 41 articles, including 47 cases, have been reported in the English literature. The mean age was 55.7 years (range 20-87 years), with 45% of patients being male. Mean tumor size was 6.2 cm (range 1-20 cm). Tumor location was the head (40%), head and body (6%), body (21%), body and tail (15%), tail (4%), and uncinate process (13%). Thirty-four percent of patients exhibited solid tumors and 60% of patients exhibited cystic tumors. Treatment included pancreaticoduodenectomy (32%), distal pancreatectomy (21%), enucleation (15%), unresectable (4%), refused operation (2%) and the detail of resection was not specified in 26% of patients. No patients died of disease with a mean follow-up of 15.7 mo (range 3-65 mo), although 5 (11%) patients had a malignancy. The tumor size was significantly related to malignant tumor (13.8 ± 6.2 cm for malignancy *vs* 5.5 ± 4.4 cm for benign, $P = 0.001$) and cystic formation (7.9 ± 5.9 cm for cystic tumor *vs* 3.9 ± 2.4 cm for solid tumor, $P = 0.005$). The preoperative diagnosis of pancreatic schwannoma remains difficult. Cystic pancreatic schwannomas should be considered in the differential diagnosis of cystic neoplasms and pseudocysts. In our case, intraoperative frozen section confirmed the diagnosis of a schwannoma. Simple enucleation may be adequate, if this is possible.

Key words: Pancreatic schwannoma; Pancreas; Schwannoma; Neurinoma; Resection; Imaging; Enucleation; Prognosis; Cystic

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INTRODUCTION

Pancreatic schwannomas are rare neoplasms that originate from Schwann cells. The Schwann cells line the nerve sheath and can generate either schwannoma or neurofibroma[1]. Schwannoma usually occur in the extremities, but can also be found in the trunk, head and neck, retroperitoneum, mediastinum, pelvis and rectum[2,3]. Pancre-

atic schwannomas are even more unusual neoplasms that affect adults with an equal gender distribution[4,5]. These tumors vary considerably in size and approximately two-thirds are reported to undergo degenerative changes including cyst formation, calcification, hemorrhage, hyalinization and xanthomatous infiltration[4,5]. As a result, they may radiographically mimic cystic pancreatic lesions (e.g., mucinous cystic neoplasms, solid and pseudopapillary neoplasms, serous cystic neoplasms, and pseudocysts).

Only 47 cases have been reported in the English literature in the last three decades[2-42]. In this report, we present a case of pancreatic schwannoma and provide a pertinent review of literature with emphasis on clinical presentation, diagnosis, treatment options, and outcome.

CASE REPORT

A 64-year-old previously healthy woman was incidentally discovered to have a cystic tumor in the pancreas during an ultrasound examination for a health check. She was referred to our institution for further investigation. The abdominal physical examination did not detect any marked finding and all laboratory data were normal, including tumor markers. The computed tomography (CT) scan demonstrated a well-encapsulated tumor, which was composed of solid and cystic areas (Figure 1), and neither liver mass nor peripancreatic lymph node swelling was detected. Magnetic resonance imaging (MRI) showed a mass, with hypointensity on T1-weighted images and hyperintensity on T2-weighted images. Magnetic resonance cholangiopancreatography (MRCP) showed a hyperintense mass in the pancreatic head with no dilatation of the main pancreatic duct. Endoscopic retrograde cholangiopancreatography revealed no communication between cystic tumor and pancreatic duct. Endoscopic ultrasonography showed that the tumor was composed of cystic part and solid part. We performed surgery under the diagnosis of cystic tumor of the pancreas as mucinous cystic tumor, solid pseudo papillary tumor or gastrointestinal stromal tumor. The laparotomy disclosed a well-encapsulated 4-cm mass in the uncinate process of the pancreas that had no signs of inflammation. Intraoperative ultrasound confirmed a solitary mass composed of solid and cystic components (Figure 2). The mass was enucleated and an intraoperative frozen section demonstrated a benign schwannoma. No further resection was performed based on these findings. The tumor was 4 cm × 4 cm × 3 cm in size, and was composed of a mixture of solid and hemorrhage areas. On microscopic examination, the tumor was composed of spindle cells strongly positive for S-100 proteins and foci of hemorrhage (hematoxylin and eosin, × 100) (Figure 3). The tumor cells were negative for smooth muscle actin and CD-34. The tumor was therefore histologically diagnosed as benign schwannoma. The patient was discharged uneventfully on postoperative day 17. At a 65-month follow up after resection, the patient is doing well without any recurrent disease.

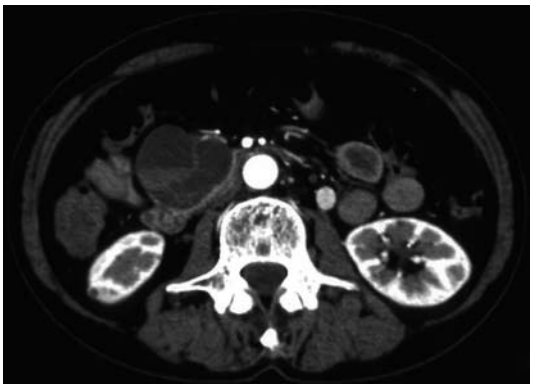

Figure 1 Contrast-enhanced computed tomography scan obtained in the arterial phase showing a multilocular cystic mass in the uncinate process of the pancreas. No pancreatic ductal dilatation or invasion into adjacent arteries or portal vein are identified.

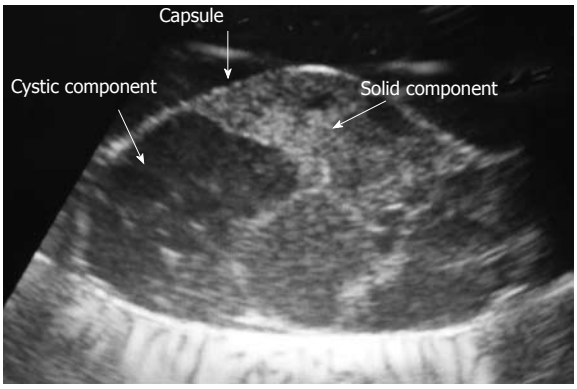

Figure 2 Intraoperative ultrasound showing the well-encapsulated pancreatic mass that is composed of solid and cystic components.

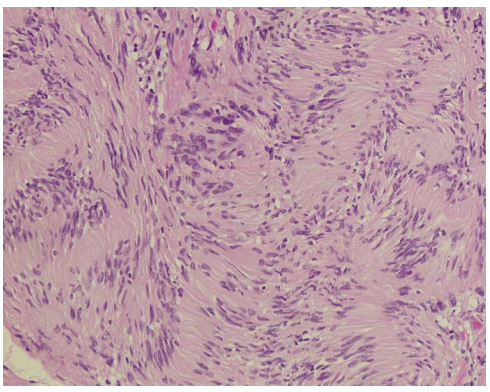

Figure 3 Microscopic examination demonstrating spindle cells without nuclear atypism (HE x 100). Immunohistochemical staining for S-100 protein was positive. HE: Hematoxylin and eosin.

DISCUSSION

A review of the patient's chart was performed along with a review of English-language articles using a PubMed search for the last three decades. We found 41 articles including 47 patients with pancreatic schwannoma. Details of the cases are summarized in Table 1 along with the current patient. Table 2 summarizes the important

Table 1 Summary of cases with pancreatic schwannoma

Author	Year	Sex	Age	Presenting symptoms	Size (cm)	Location	Solid/cystic by image	Treatment	Histology	Follow-up (mo)
Current case	2010	F	64	Asymptomatic	4.0	Uncinate	Solid and cystic	Enucleation	Benign	65
Dorsey *et al*[6]	2010	F	54	Abdominal pain, weight loss	1.4	Head	Solid	NA	Benign	NA
Stojanovic *et al*[7]	2010	F	24	Abdominal pain, dyspepsia, weight loss, palpable tumor	18.0	Body/tail	Cystic	DP + transverse colon	Malignant	28
Suzuki *et al*[8]	2010	F	66	Asymptomatic	3.0	Body	Solid and cystic	DP	Benign	24
Aggarwal *et al*[9]	2010	M	20	Upper abdominal discomfort	3.0	Head	NA	Enucleation	Benign	NA
Ohshima *et al*[10]	2010	F	32	Back pain	4.0	Head	Solid and cystic	PD	Benign	NA
Mummadi *et al*[11]	2009	M	35	Epigastric pain	7.0	Body	Solid and cystic	NA	Benign	6
Gupta *et al*[5]	2009	F	56	Asymptomatic	8.3	Head/body	Cystic	PD	Benign	NA
Li *et al*[12]	2009	M	37	Asymptomatic	16.0	Head	Solid and Cystic	PD	Benign	NA
Tafe *et al*[13]	2008	M	46	Abdominal pain	11.0	Body/tail	Cystic	DP	Benign	NA
Hirabayashi *et al*[14]	2008	M	51	Asymptomatic	6.0	Tail	Cystic	DP	Benign	NA
Okuma *et al*[15]	2008	F	71	Epigastric pain	4.0	Body	Solid and cystic	DP	Benign	NA
Tofigh *et al*[16]	2008	M	54	Epigastric pain, weight loss, nausea, intermittent jaundice	3.0	Head	Solid (by specimen)	PD	Benign	
Fasanella *et al*[17]	2007	M	36	Abdominal discomfort	3.6	Uncinate	Cystic	NA	Benign	NA
Di Benedetto *et al*[18]	2007	M	42	Asymptomatic	2.5	Body	Solid	DP	Benign	NA
Yu *et al*[19]	2006	M	72	Upper abdominal pain	1.0	Head/body	Solid	NA	Benign	NA
Wu *et al*[20]	2005	M	71	Epigastric pain, decreased appetite	1.5	Head	Cystic	Enucleation	Benign	10
Novellas *et al*[21]	2005	F	46	Asthenia, weight loss, empyema	3.0	Head	Solid	PD	Benign	24
Soumaoro *et al*[22]	2005	F	64	Asymptomatic	2.5	Head	Solid	Enucleation	Benign	24
Bui *et al*[23]	2004	F	69	Abdominal pain	5.0	Head	Solid	Unresectable	NA[1]	NA
Akiyoshi *et al*[24]	2004	F	67	Asymptomatic	5.0	Head	Cystic	PD	Benign	43
Von Dobschuetz *et al*[25]	2004	F	55	Asymptomatic	8.0	Head	Cystic	PD + PV reconstruction	Benign	10
Paranjape *et al*[4]	2004	F	77	Upper abdominal pain, weight loss	3.5	Body	Solid	Enucleation	Benign	3
Tan *et al*[26]	2003	F	46	Right upper quadrant pain	2.2	Head	Solid and Cystic	PD	Benign	NA
Almo *et al*[2]	2001	F	73	Abdominal pain, nausea, vomiting	3.0	Head	Cystic	PD	Benign	17
Almo *et al*[2]	2001	F	47	Abdominal pain, back pain	5.5	Head	Solid	PD	Benign	14
Lee *et al*[27]	2001	F	63	Upper abdominal pain	10.0	Tail	Cystic	DP	Benign	6
Morita *et al*[28]	1999	F	50	Upper abdominal pain	9.5	Body/tail	Cystic	DP	Benign	7
Brown *et al*[29]	1998	M	52	Asymptomatic	5.5	Body	Cystic	Resection[2]	Benign	NA
Brown *et al*[29]	1998	M	69	Asymptomatic	6.0	Head	Cystic	PD	Benign	NA
Hsiano *et al*[30]	1998	F	70	Palpable tumor	18.0	Body/tail	Cystic	Resection[2]	Benign	24
Feldman *et al*[31]	1997	M	63	Asymptomatic	2.5	Body	Solid	Enucleation	Benign	NA
Feldman *et al*[31]	1997	F	54	Abdominal pain	2.0	Uncinate	Solid	Enucleation	Benign	22
Ferrozzi *et al*[32]	1995	M	47	Right-sided abdominal pain	3.5	Body	NA	DP	Benign	48
Ferrozzi *et al*[32]	1995	M	63	Abdominal pain	NA	Body	Cystic	NA	Benign	NA
Ferrozzi *et al*[32]	1995	F	68	Upper abdominal pain	NA	Head/body	Cystic	NA	Benign	6
Sugiyama *et al*[33]	1995	M	41	Asymptomatic	1.5	Uncinate	Cystic	PD	Benign	NA
Steven *et al*[34]	1994	M	59	Asymptomatic	4.0	Uncinate	Solid	PD	Benign	10
Melato *et al*[35]	1993	M	87	Upper abdominal pain	20.0	Body/tail	Cystic	NA	Benign	NA
David *et al*[3]	1993	M	46	Right sided abdominal pain	6.0	Uncinate	Cystic	NA	Benign	NA
Urban *et al*[36]	1992	F	56	Right sided hip pain	4.0	Body	Cystic	DP	Benign	NA
Burd *et al*[37]	1992	M	73	Right upper quadrant abdominal pain	2.0	Body/tail	Solid	NA	Benign	NA
Coombs *et al*[38]	1990	F	74	Anemia, melena	7.0	Head	Solid with necrotic center	NA	Malignant	NA
Liessi *et al*[39]	1990	F	75	Abdominal pain	7.0	Head	Solid	Not resected	Benign	7
Walsh *et al*[40]	1989	F	35	Abdominal pain, melena, anemia	NA	Head	NA	PD	Malignant	24
Eggermont *et al*[41]	1987	F	40	Upper abdominal pain, jaundice, weight loss	10.0	Head	Solid with necrotic center	PD	Malignant	9
Moller-Pederson *et al*[42]	1982	M	60	Back pain, weight loss	20.0	Body/tail	Cystic	Unresectable	Malignant	4

M: Male; F: Female; NA: Not available. [1]Unresectable because of encasing the superior mesenteric artery and the portal vein, although the malignant finding was not confirmed by histopathology; [2]No specific operation documented.

Table 2 Summary of clinicopathological data from all 47 cases of pancreatic schwannoma

	n (%) or mean ± SD (range)
Age (yr)	55.7 ± 15.1 (20-87)
Sex (male/female), (male %)	21/26 (45%)
Symptoms[1]	
Asymptomatic	14 (30%)
Symptomatic	
Abdominal pain	27 (57%)
Weight loss	6 (13%)
Back pain	3 (6%)
Nausea/vomiting	2 (4%)
Abdominal mass	2 (4%)
Anemia	2 (4%)
Melena	2 (4%)
Jaundice	1 (4%)
Location	
Head	19 (40%)
Head/body	3 (6%)
Body	10 (21%)
Body/tail	7 (15%)
Tail	2 (4%)
Uncinate process	6 (13%)
Mean size (cm), (*n* = 44)	6.2 ± 5.1 (1-20)
Operation	
Pancreaticoduodenectomy[2]	15 (32%)
Distal pancreatectomy[3]	10 (21%)
Enucleation	7 (15%)
Unresectable	2 (4%)
Refused	1 (2%)
Not specified	12 (26%)
Histology	
Malignant	5 (11%)
Benign	41 (87%)
Not specified	1 (2%)
Nature of tumor	
Solid	16 (34%)
Cystic	28 (60%)
Not specified	3 (6%)
Mean follow-up months (*n* = 23)	18.9 ± 15.7 (3-65)
Died of disease	0 (0%)

[1]Patients had several symptoms; [2]One patient underwent resection of portal vein; [3]One patient underwent resection of transverse colon.

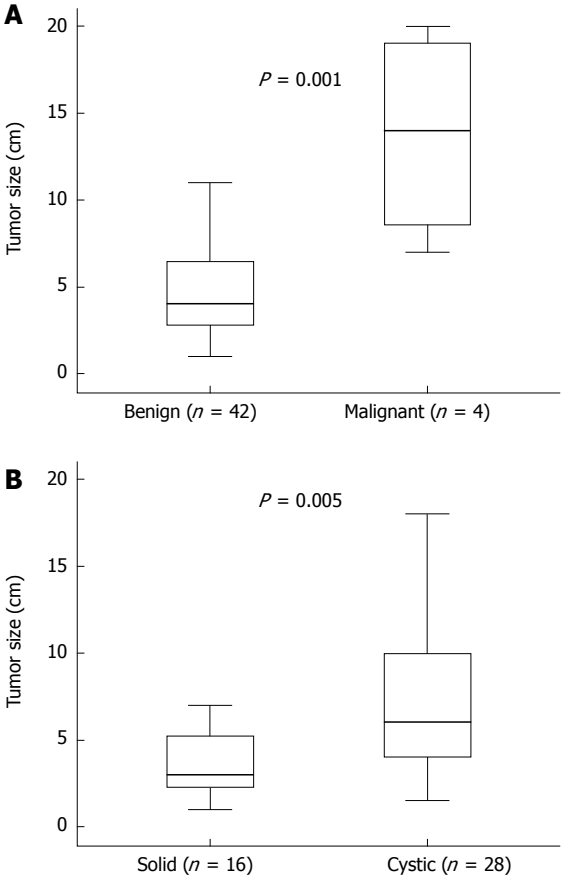

Figure 4 Analysis for relation between tumor size and malignant potential and tumor nature (solid or cystic) in all 47 cases of pancreatic schwannoma. A: Relationship between tumor size and malignancy. Larger tumor size is related to malignant tumor (13.8 ± 6.2 cm for malignancy *vs* 5.5 ± 4.4 cm for benign, $P = 0.001$); B: Relationship between tumor size and nature of tumor. Larger tumor size is related to cystic degeneration (13.8 ± 6.2 cm for cystic tumor *vs* 5.5 ± 4.4 cm for solid tumor, $P = 0.005$).

available facts regarding all patients. We examined the correlation between tumor size and malignancy, as well as tumor size and cystic degeneration. Continuous data are presented as mean ± standard deviation and range. Student *t*-test was used for all comparisons among continuous variables. A $P < 0.05$ was considered statistically significant.

A PubMed search of the literature indicated 41 reports including 47 patients with pancreatic schwannoma in the English literature. Details of all the 47 cases are summarized in Table 1. Table 2 summarizes the important available clinicopathological factors. The mean age of the patients was 55.7 ± 15.1 years (range 20-87 years) and the male-female ratio was 21:26. Thirty percent of patients were asymptomatic and 70% of patients were symptomatic. Symptoms included abdominal pain (57%), weight loss (13%), back pain (6%), nausea/vomiting (4%), abdominal mass (4%), melena (4%), and jaundice (4%). The symptoms did not correlate with tumor size and tumor location. The lesion was located in the pancreas head in 19 patients (40%), head and body in 3 patients (6%), body in 10 patients (21%), body and tail in 7 (15%), tail in 2 patients (4%), and uncinate process in 6 patients (13%). Mean tumor size was 6.2 ± 5.1 cm (range 1-20 cm). Treatment included pancreaticoduodenectomy for 15 patients (32%) including one portal vein reconstruction, distal pancreatectomy for 10 patients (21%) including combined transverse colon resection, enucleation for 7 patients (15%), unresectable for 2 patients (4%), refused operation for 1 patient (2%) and the detail of resection was not specified in 12 patients (26%). Enucleation was performed for 7 patients, and out of these, 3 lesions were located in the head, 2 lesions were in the uncinate process and 2 lesions were in the body. The mean tumor size in the patients who underwent enucleation was 2.7 cm (range 1.5-4.0 cm). Regarding gross appearance, 34% of patients exhibited solid tumors and 60% of patients exhibited cystic tumors. No patient died of disease with a follow-up of 15.7 mo (range 3-65 mo), although 4 (9%) patients had a malignancy. The tumor size was related to malignant tumor (13.8 ± 6.2 cm for malignancy *vs* 5.5 ± 4.4 cm for benign, $P = 0.001$) (Figure 4A) and cystic for-

mation (7.9 ± 5.9 cm for cystic tumor *vs* 3.9 ± 2.4 cm for solid tumor, $P = 0.005$) (Figure 4B).

In 1910, Verocay reported a schwannoma as a true neoplasm which originated from Schwann cells, and which did not contain neuroganglion cells[1]. Since then, schwannomas have become well known as benign spindle cell tumors derived from Schwann cells that line the nerve sheaths. Schwannomas usually occur in the extremities, but can also be found in the trunk, head and neck, retroperitoneum, mediastinum, pelvis and rectum[2-4]. Pancreatic schwannomas are rare neoplasms that arise from either autonomic sympathetic or parasympathetic fibers, both of which course through the pancreas as branches of the vagus nerve[2-4].

Microscopically, a typical schwannoma is composed of 2 areas, namely Antoni A and Antoni B areas. The Antoni A area is hypercellular and characterized by closely packed spindle cells with occasional nuclear palisading and Verocay bodies, whereas the Antoni B area is hypocellular and is occupied by loosely arranged tumor cells[43]. Most of the pancreatic schwannomas reported had both Antoni A and Antoni B areas in various proportions. Degenerative or cystic changes such as calcification or hemorrhage are often recognized in the Antoni B area. These changes result from vascular thrombosis and subsequent necrosis[43]. Cystic pancreatic schwannomas can mimic the whole spectrum of cystic pancreatic lesions including: intraductal mucinous-papillary neoplasms, mucinous cystic neoplasms, serous cystic neoplasms, solid and pseudo-papillary neoplasms, lymphangiomas, and pancreatic pseudocysts. Immunohistochemically, schwannomas stain strongly positive for S-100 protein, vimentin and CD 56, while negative for other tumor markers including cytokeratin AE1/AE3, desmin, smooth muscle myosin, CD 34 and CD 117[43].

The symptoms of the reported patient cases of pancreatic schwannoma vary. Seventy percent of patients were symptomatic. Abdominal pain was the most common symptom reported (57%). Symptoms such as back pain (6%), nausea/vomiting (4%), weight loss (13%), melena (4%) and jaundice (4%) have been also reported. Thirty percent of patients were asymptomatic and the lesions were incidentally discovered on CT scans performed for other reasons.

The preoperative diagnosis of pancreatic schwannoma is very difficult, especially in cystic schwannomas. Suzuki *et al*[8] reviewed imaging features of pancreatic schwannomas. The most characteristic feature on CT scan was the presence of an area of low density and/or cystic images reflecting the Antoni B component or degenerative cystic areas of the schwannoma. Contrast-enhanced CT scan showed the difference between the Antoni A and the Antoni B areas based on their vascularity, i.e., well-enhanced areas corresponding to Antoni A, and unenhanced areas corresponding to Antoni B. The CT findings correlated well with pathological features[8,19]. The MRI findings usually showed hypointensity on T1-weighted images and hyperintensity on T2-weighted images[21]. However, other pancreatic tumors often share those imaging features, and differential diagnoses should always be considered. Ultrasound-guided Fine Needle Aspiration (EUS-FNA) biopsy has been used increasingly commonly at many institutions. This procedure may be useful for accurate preoperative diagnosis. Cytologically, schwannomas are characteristically composed of spindle-shaped cells, which possess indistinct cytoplasmic borders and wavy nuclei embedded in a fibrillary and occasionally myxoid or collagenous matrix. The Antoni A (cohesive cellular clusters) and Antoni B (loosely cohesive or poorly cellular sheets) areas are occasionally found. Immunohistochemical staining is useful for accurate diagnosis of schwannoma[12,14]. It is diffusely and strongly positive for S-100 protein. There has only been one previous report of pancreatic schwannoma diagnosed preoperatively by EUS-FNA cytology combined with immunohistochemistry[12].

Although malignant pancreatic schwannomas have been reported in 5 articles[7,38,40-42], in 3 of 5 the methods of diagnosing malignancy were inconsistent, as some previous reports pointed out[4,5]. Immunohistochemical examination was not used or was not available in these 3 patients. Walsh and Bradspigel[40] described a case of pancreatic schwannoma eroding into the bowel wall and presenting with gastrointestinal bleeding that mimicked a recurrently bleeding duodenal ulcer. Another two patients reported had disease associated with von Recklinghausen's disease[41,42]. These could represent misdiagnosed neurofibromas that underwent malignant degeneration[4,5]. Stojanovic *et al*[7] reported malignant pancreatic schwannoma with node metastasis and infiltrating serosa of transverse colon. This tumor was confirmed using immunohistochemical examination. This may be the first definite report of malignant schwannoma with subsequent radical resection.

Since malignant transformation of pancreatic schwannomas is uncommon, simple enucleation is usually sufficient. A review of the treatment showed that the most common resection was pancreaticoduodenectomy (32%), followed by distal pancreatectomy (21%) and enucleation (15%). This result may account for the difficulty in accurate diagnosis of pancreatic schwannoma and relate to larger size of this tumor. Enucleation was performed for 7 patients for whom 3 lesions were located in the head, 2 lesions were in the uncinate process and 2 lesions were in the body. The mean tumor size of the patients who underwent enucleation was 2.7 cm (range 1.5-4.0 cm). Intraoperative consultation with the pathologist was carried out in most of the enucleated cases. An intraoperative frozen section should be performed, as it helps to establish the diagnosis of a benign schwannoma and avoid more radical resection. Large tumors, tumors involving portal vein, ampulla, or splenic hilum, may require a more radical resection than simple enucleation.

The present report shows the correlation between tumor size and malignant formation (Figure 4A), and tumor size and cystic degeneration (Figure 4B). Malignant schwannomas were more likely to be larger-sized compared to many other tumors. On the other hand, the par-

ticular feature of pancreatic schwannoma was that larger tumor size was related to cystic degeneration, as shown in Figure 4B. Cystic degeneration could make it difficult to diagnose pancreatic schwannoma preoperatively, because of mimicking other cystic neoplasms. Caution should be applied when diagnosing cystic neoplasm. An intraoperative frozen section may help to establish the diagnosis of a schwannoma and avoid more radical resection. To our knowledge, the present report is the first to analyze the relation among tumor size, malignant formation and cystic degeneration. Our results suggest that pancreatic schwannoma might be resected even though diagnosed preoperatively, because if schwannomas are smaller, enucleation should be oncologically adequate. However, when tumors become larger with associated bleeding risk, more invasive resection such a PD or DP might be necessary. In particular, in cases of tumors more than 10 cm in size, we should pay special attention to malignant degeneration and should perform a more extended resection. To avoid extended resection, earlier resection and accurate diagnosis are very important.

In conclusion, pancreatic schwannomas deserve attention with regard to the differential diagnosis of pancreatic lesions. Preoperative diagnosis is very difficult. Simple enucleation is adequate if this is possible to achieve. Intraoperative frozen section is useful to diagnose schwannoma.

REFERENCES

1 **Verocay J**. Zur Kenntnis der "Neurofibrome". *Beitr Pathol Anat Allg Pathol* 1910; **48**: 1-69
2 **Almo KM,** Traverso LW. Pancreatic schwannoma: an uncommon but important entity. *J Gastrointest Surg* 2001; **5**: 359-363
3 **David S**, Barkin JS. Pancreatic schwannoma. *Pancreas* 1993; **8**: 274-276
4 **Paranjape C**, Johnson SR, Khwaja K, Goldman H, Kruskal JB, Hanto DW. Clinical characteristics, treatment, and outcome of pancreatic Schwannomas. *J Gastrointest Surg* 2004; **8**: 706-712
5 **Gupta A**, Subhas G, Mittal VK, Jacobs MJ. Pancreatic schwannoma: literature review. *J Surg Educ* 2009; **66**: 168-173
6 **Dorsey F**, Taggart MW, Fisher WE. Image of the month. Pancreatic schwannoma. *Arch Surg* 2010; **145**: 913-914
7 **Stojanovic MP**, Radojkovic M, Jeremic LM, Zlatic AV, Stanojevic GZ, Jovanovic MA, Kostov MS, Katic VP. Malignant schwannoma of the pancreas involving transversal colon treated with en-bloc resection. *World J Gastroenterol* 2010; **16**: 119-122
8 **Suzuki S**, Kaji S, Koike N, Harada N, Hayashi T, Suzuki M, Hanyu F, Ban S. Pancreatic schwannoma: a case report and literature review with special reference to imaging features. *JOP* 2010; **11**: 31-35
9 **Aggarwal G**, Satsangi B, Shukla S, Lahoti BK, Mathur RK, Maheshwari A. Rare asymptomatic presentations of schwannomas in early adolescence: three cases with review of literature. *Int J Surg* 2010; **8**: 203-206
10 **Oshima M**, Yachida S, Suzuki Y. Pancreatic schwannoma in a 32-year-old woman mimicking a solid-pseudopapillary neoplasm. *Clin Gastroenterol Hepatol* 2010; **8**: e1-e2
11 **Mummadi RR**, Nealon WH, Artifon EL, Fleming JB, Bhutani MS. Pancreatic Schwannoma presenting as a cystic lesion. *Gastrointest Endosc* 2009; **69**: 341; discussio 341
12 **Li S**, Ai SZ, Owens C, Kulesza P. Intrapancreatic schwannoma diagnosed by endoscopic ultrasound-guided fine-needle aspiration cytology. *Diagn Cytopathol* 2009; **37**: 132-135
13 **Tafe LJ**, Suriawinata AA. Cystic pancreatic schwannoma in a 46-year-old man. *Ann Diagn Pathol* 2008; **12**: 296-300
14 **Hirabayashi K**, Yasuda M, Umemura S, Itoh H, Itoh J, Yazawa N, Imaizumi T, Osamura RY. Cytological features of the cystic fluid of pancreatic schwannoma with cystic degeneration. A case report. *JOP* 2008; **9**: 203-208
15 **Okuma T**, Hirota M, Nitta H, Saito S, Yagi T, Ida S, Okamura S, Chikamoto A, Iyama K, Takamori H, Kanemitsu K, Baba H. Pancreatic schwannoma: report of a case. *Surg Today* 2008; **38**: 266-270
16 **Tofigh AM**, Hashemi M, Honar BN, Solhjoo F. Rare presentation of pancreatic schwannoma: a case report. *J Med Case Reports* 2008; **2**: 268
17 **Fasanella KE**, Lee KK, Kaushik N. Clinical challenges and images in GI. Benign schwannoma of the pancreatic head. *Gastroenterology* 2007; **132**: 489, 830
18 **Di Benedetto F**, Spaggiari M, De Ruvo N, Masetti M, Montalti R, Quntini C, Ballarin R, Di Sandro S, Costantini M, Gerunda GE. Pancreatic schwannoma of the body involving the splenic vein: case report and review of the literature. *Eur J Surg Oncol* 2007; **33**: 926-928
19 **Yu RS**, Sun JZ. Pancreatic schwannoma: CT findings. *Abdom Imaging* 2006; **31**: 103-105
20 **Wu YL**, Yan HC, Chen LR, Chen J, Gao SL, Li JT. Pancreatic benign schwannoma treated by simple enucleation: case report and review of literature. *Pancreas* 2005; **31**: 286-288
21 **Novellas S**, Chevallier P, Saint Paul MC, Gugenheim J, Bruneton JN. MRI features of a pancreatic schwannoma. *Clin Imaging* 2005; **29**: 434-436
22 **Soumaoro LT**, Teramoto K, Kawamura T, Nakamura N, Sanada T, Sugihara K, Arii S. Benign schwannoma of the pancreas. *J Gastrointest Surg* 2005; **9**: 288-290
23 **Bui TD**, Nguyen T, Huerta S, Gu M, Hsiang D. Pancreatic schwannoma. A case report and review of the literature. *JOP* 2004; **5**: 520-526
24 **Akiyoshi T**, Ueda Y, Yanai K, Yamaguchi H, Kawamoto M, Toyoda K, Hayashi T, Ohuchida J. Melanotic schwannoma of the pancreas: report of a case. *Surg Today* 2004; **34**: 550-553
25 **von Dobschuetz E**, Walch A, Werner M, Hopt UT, Adam U. Giant ancient schwannoma of pancreatic head treated by extended pancreatoduodenectomy. *Pancreatology* 2004; **4**: 505-508
26 **Tan G**, Vitellas K, Morrison C, Frankel WL. Cystic schwannoma of the pancreas. *Ann Diagn Pathol* 2003; **7**: 285-291
27 **Lee JS**, Kim HS, Jung JJ, Han SW, Kim YB. Ancient schwannoma of the pancreas mimicking a cystic tumor. *Virchows Arch* 2001; **439**: 697-699
28 **Morita S**, Okuda J, Sumiyoshi K, Taketani M, Moriguchi A, Katsu K, Tanigawa N. Pancreatic Schwannoma: report of a case. *Surg Today* 1999; **29**: 1093-1097
29 **Brown SZ**, Owen DA, O'Connell JX, Scudamore CH. Schwannoma of the pancreas: a report of two cases and a review of the literature. *Mod Pathol* 1998; **11**: 1178-1182
30 **Hsiao WC**, Lin PW, Chang KC. Benign retroperitoneal schwannoma mimicking a pancreatic cystic tumor: case report and literature review. *Hepatogastroenterology* 1998; **45**: 2418-2420
31 **Feldman L**, Philpotts LE, Reinhold C, Duguid WP, Rosenberg L. Pancreatic schwannoma: report of two cases and review of the literature. *Pancreas* 1997; **15**: 99-105
32 **Ferrozzi F**, Bova D, Garlaschi G. Pancreatic schwannoma: report of three cases. *Clin Radiol* 1995; **50**: 492-495
33 **Sugiyama M**, Kimura W, Kuroda A, Muto T. Schwannoma arising from peripancreatic nerve plexus. *AJR Am J Roentgenol* 1995; **165**: 232
34 **Steven K**, Burcharth F, Holm N, Pedersen IK. Single stage pancreaticoduodenectomy (Whipple's procedure), radical cystectomy and bladder substitution with the urethral Kock reservoir. Case report. *Scand J Urol Nephrol* 1994; **28**: 199-200

35 **Melato M**, Bucconi S, Marus W, Spivach A, Perulli A, Mucelli RP. The schwannoma: an uncommon type of cystic lesion of the pancreas. *Ital J Gastroenterol* 1993; **25**: 385-387
36 **Urban BA**, Fishman EK, Hruban RH, Cameron JL. CT findings in cystic schwannoma of the pancreas. *J Comput Assist Tomogr* 1992; **16**: 492-493
37 **Burd DA**, Tyagi G, Bader DA. Benign schwannoma of the pancreas. *AJR Am J Roentgenol* 1992; **159**: 675
38 **Coombs RJ**. Case of the season. Malignant neurogenic tumor of duodenum and pancreas. *Semin Roentgenol* 1990; **25**: 127-129
39 **Liessi G**, Barbazza R, Sartori F, Sabbadin P, Scapinello A. CT and MR imaging of melanocytic schwannomas; report of three cases. *Eur J Radiol* 1990; **11**: 138-142
40 **Walsh MM**, Brandspigel K. Gastrointestinal bleeding due to pancreatic schwannoma complicating von Recklinghausen's disease. *Gastroenterology* 1989; **97**: 1550-1551
41 **Eggermont A**, Vuzevski V, Huisman M, De Jong K, Jeekel J. Solitary malignant schwannoma of the pancreas: report of a case and ultrastructural examination. *J Surg Oncol* 1987; **36**: 21-25
42 **Móller Pedersen V**, Hede A, Graem N. A solitary malignant schwannoma mimicking a pancreatic pseudocyst. A case report. *Acta Chir Scand* 1982; **148**: 697-698
43 **Weiss SW**, Langloss JM, Enzinger FM. Value of S-100 protein in the diagnosis of soft tissue tumors with particular reference to benign and malignant Schwann cell tumors. *Lab Invest* 1983; **49**: 299-308

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CASE REPORT

Esophageal combined carcinomas: Immunohoistochemical and molecular genetic studies

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Abstract

Primary esophageal combined carcinoma is very rare. The authors herein report 2 cases. Case 1 was a combined squamous cell carcinoma and small cell carcinoma, and case 2 was a combined squamous cell carcinoma, adenocarcinoma, and small cell carcinoma. Case 1 was a 67-year-old man with complaints of dysphagia. Endoscopic examination revealed an ulcerated tumor in the middle esophagus, and 6 biopsies were obtained. All 6 biopsies revealed a mixture of squamous cell carcinoma and small cell carcinoma. Both elements were positive for cytokeratin, epithelial membrane antigen, and p53 protein, and had high Ki-67 labeling. The small cell carcinoma element was positive for synaptophysin, CD56, KIT, and platelet-derived growth factor-α (PDGFRA), while the squamous cell carcinoma element was not. Genetically, no mutations of *KIT* and *PDGFRA* were recognized. The patient died of systemic carcinomatosis 15 mo after presentation. Case 2 was a 74-year-old man presenting with dysplasia. Endoscopy revealed a polypoid tumor in the distal esophagus. Seven biopsies were taken, and 6 showed a mixture of squamous cell carcinoma, small cell carcinoma, and adenocarcinoma. The 3 elements were positive for cytokeratins, epithelial membrane antigen, and p53 protein, and had high Ki-67 labeling. The adenocarcinoma element was positive for mucins. The small cell carcinoma element was positive for CD56, synaptophysin, KIT, and PDGFRA, but the other elements were not. Mutations of KIT and PDGFRA were not recognized. The patient died of systemic carcinomatosis 7 mo after presentation. These combined carcinomas may arise from enterochromaffin cells or totipotential stem cell in the esophagus or transdifferentiation of one element to another. A review of the literature was performed.

Key words: Esophagus; Combined carcinoma; Histopathology; Immunohistochemistry; Molecular genetics

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INTRODUCTION

Combined esophageal carcinomas are very rare and interesting tumors. A full review of the English literature revealed 24 reporting combined carcinoma of the esophagus[1-24]. Most were small cell carcinomas, and a few were non-small cell carcinomas[1-24]. The author herein reports 2 cases of combined carcinoma of the esophagus. One case is a combined squamous cell carcinoma and small cell carcinoma, and another case is a combined squamous cell carcinoma, adenocarcinoma, and small cell carcinoma

CASE REPORT

Case 1

A 67-year-old man was admitted to our hospital with dysphagia. An endoscopic examination revealed an ulcerated tumor (3 cm × 4 cm × 3 cm) in the middle esophagus (Figure 1A), and 6 biopsies were obtained. All 6 biopsies revealed a mixture of squamous cell carcinoma (Figure 1B) and small cell carcinoma (Figure 1C). The squamous element was composed of malignant cells arranged in a layer with focal keratinization (cancer pearls). The small cell carcinoma element consisted of malignant small cells with hyperchromatic nuclei, nuclear molding, absent nucleoli, and very scant cytoplasm. There was a gradual merging of the 2 elements.

The authors performed an immunohistochemical study using Dako Envision method, as previously described[25,26]. The immunohistochemical antibodies used were as follows: cytokeratins (AE1/3, Dako; CAM5.2 Bekton-Dickinson, CA, United States), epithelial membrane antigen (E29, Dako), neuron-specific enolase (BBS/NC/VI-H14, Dako), chromogranin (DAK-A3, Dako), synaptophysin (polyclonal, Dako), CD56 (UJ13A, Dako), p53 protein (DO-7, Dako), Ki-67 (MIB-1, Dako), KIT (polyclonal, Dako), and platelet derived growth factor receptor-α (PDGFRA) (polyclonal, Santa Cruz, CA, United States). The squamous cell carcinoma element was positive for cytokeratin, epithelial membrane antigen, p53 protein, and Ki-67 antigen (57% labeled), but negative for other antigens examined. The small cell carcinoma element was positive for cytokeratin (Figure 1D), p53 protein, Ki-67 (96% labeled), synaptophysin (Figure 1E), CD56, and chromogranin, KIT (Figure 1F), and PDGFRA (Figure 1G).

The authors performed a molecular genetic study for *KIT* (exons 9, 11, 13 and 17) and *PDGFRA* (exons 12 and 18) genes in paraffin sections using microdissection and the polymerase chain reaction-direct sequencing method, as previously described[27-30]. There were no mutations of the *KIT* (exons 9, 11, 13 and 17) and *PDGFRA* (exons 12 and 18) genes.

The patient was diagnosed with combined carcinoma of esophagus (stage Ⅱ, T2 N0 M0). Surgery was not considered because the tumor contained small cell carcinoma. The patient was treated with cisplatin-based chemotherapy and radiation, but died of systemic carcinomatosis 15 mo after presentation.

Case 2

A 74-year-old man presented with dysplasia, and attended our hospital. An endoscopy revealed a polypoid tumor (2 cm × 2 cm × 3 cm) in the middle esophagus (Figure 2A). Seven biopsies were taken, and 6 showed a mixture of squamous cell carcinoma (Figure 2B), small cell carcinoma (Figure 2C), and adenocarcinoma (Figure 2D). The squamous cell carcinoma element showed malignant cells in a layer with focal keratinization. The small cell carcinoma element was composed of small malignant cells with hyperchromatic nuclei, inconspicuous nucleoli, and scant cytoplasm. The adenocarcinoma element showed sheet-like tumor cells with focal acinar formations, in which mucins were identified. The 3 elements were positive for cytokeratins, epithelial membrane antigen, p53 protein, and Ki-67 (labeling: squamous cell carcinoma element, 34%; adenocarcinoma element, 29%; small cell carcinoma element 87%). The squamous cell carcinoma and adenocarcinoma elements were negative for CD56, chromogranin, synaptophysin, neuron-specific elolase, KIT and PDGFRA. In contrast, the small cell carcinoma element was positive for CD56 (Figure 2E), synaptophysin, KIT (Figure 2F), and PDGFRA (Figure 2G). Mutations of KIT and PDGFRA were not found.

The patient was diagnosed with combined carcinoma of the esophagus (stage Ⅱ, T2 N1 M0). Surgery was not considered because the tumor contained small cell carcinoma. The patient received chemoradiation, but died of systemic carcinomatosis 7 mo after presentation.

DISCUSSION

The present 2 cases of combined carcinoma of the esophagus were associated with small cell carcinoma. Small cell carcinoma is diagnosed with hematoxylin and eosin (HE) staining and is defined as an undifferentiated carcinoma consisting of small cells with characteristic cellular and nuclear features, such as small-sized cells, scant cytoplasm, hyperchromatic, finely granular, and molded nuclei, and inconspicuous nucleoli, according to the World Health Organization Blue Book[31]. Neuroendocrine features are recognized in more than 90% of small cell carcinoma[31]. Squamous cell carcinoma is characterized by a squamoid cell arrangement and the presence of intercellular bridges and keratinization. Adenocarcinoma is characterized by tubular formations and the presence of mucins. Case 1 in the present study fulfilled these criteria, and was definitely combined small cell carcinoma and squamous cell carcinoma. Likewise, Case 2 was an apparently combined small cell carcinoma, squamous cell carcinoma, and adenocarcinoma. The presence of p53 protein and high Ki-67 labeling supports the above diagnosis.

In the present study, there was gradual merging of the 2 elements in case 1 and of the 3 elements in case 2. These findings may indicate that each element is derived from transdifferentiation of other elements. Traditionally, small cell carcinoma of the esophagus is thought to be derived from enterochromaffin cells or APUD cells present in the normal esophagus. Otherwise, this esophageal tumor arises from totipotent stem cells of the esophagus, as suggested by Ho *et al*[2]. The present study could not determine the histogenesis of the combined carcinomas associated with small cell carcinomas.

Most of esophageal tumors with multiple differentiation (combined carcinoma) are associated with small cell carcinoma[1-15,17-24], although basaloid cell squamous cell carcinoma also shows multiple differentiation[16]. The cel-

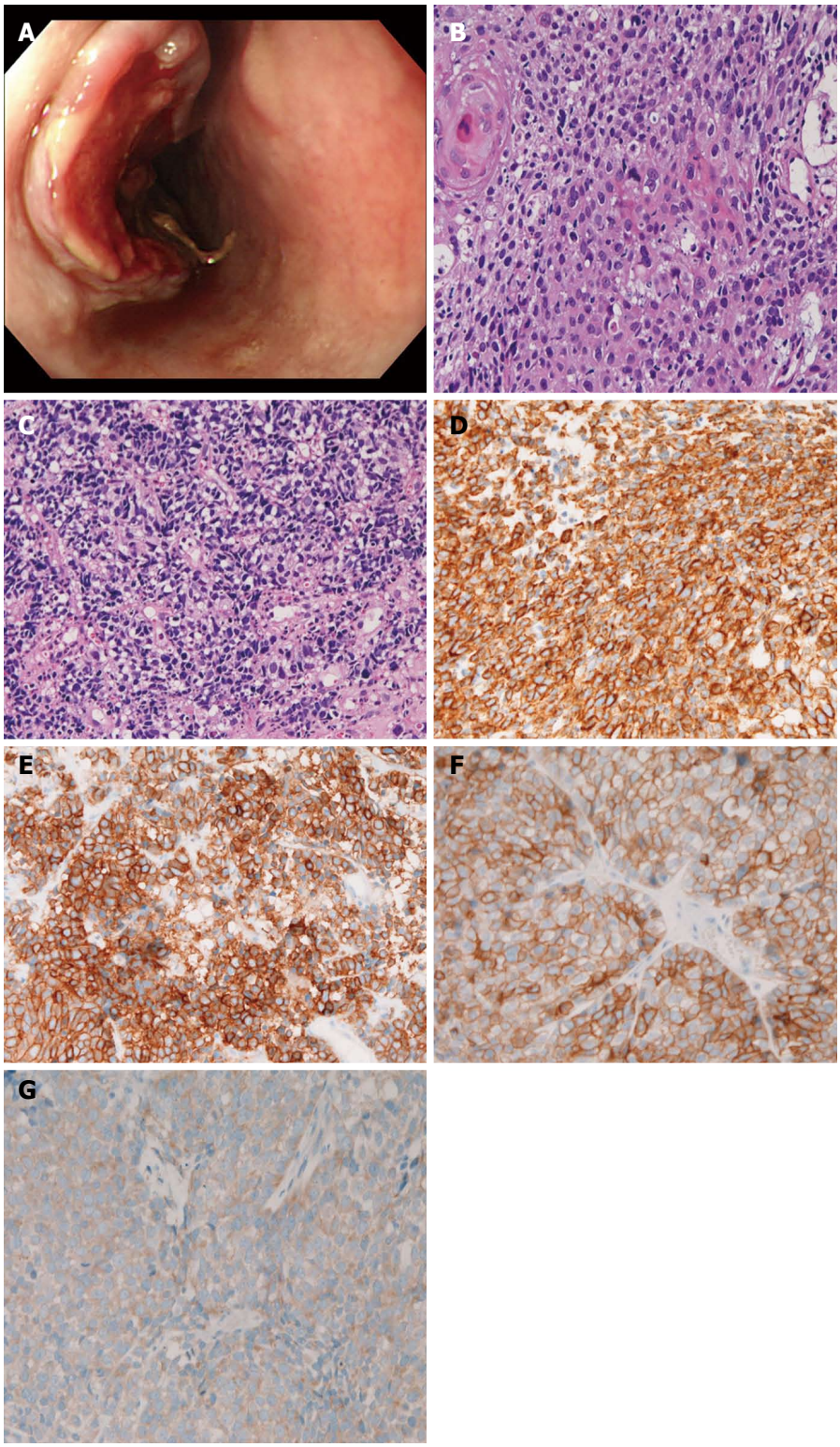

Figure 1 Case 1. A: Endoscopy. An ulcerated tumor is seen in the esophagus; B: Histology of the squamous cell carcinoma element of the esophageal tumor. Keratinization is seen [hematoxylin and eosin (HE), x 200]; C: Small cell carcinoma element of the esophageal carcinoma. The tumor cells show characteristic morphologies of small cell carcinoma (HE, x 200); D: Cytokeratins are expressed in the small cell carcinoma component (x 200); E: Synaptophysin is expressed in the small cell carcinoma component (x 200); F: KIT is expressed in the small cell carcinoma component (x 200); G: Platelet-derived growth factor-α is expressed in the small cell carcinoma component (x 200).

lular origin of small cell carcinoma is unknown. In the full review of the English literature on combined carcinomas of esophageal cancers, Rosen *et al*[1] reported an epidermoid carcinoma simulating oat cell carcinoma. Ho *et al*[2] reported that 2 of 4 cases of esophageal small cell carcinoma contained foci of squamous cell carcinoma. Reid *et al*[3] described a case of esophageal small cell carcinoma with foci of squamous cell carcinoma. Reyes *et al*[4] reported that foci of squamous cell carcinoma were seen in 4/16 esophageal small cell carcinoma. Sarma[5] mentioned that there were oat cell carcinomas with squamous cell carcinoma foci and adenocarcinoma foci. Doherty *et al*[6] reported that there were oat cell carcinomas with squamous cell carcinoma *in situ*, with squamous cell carcinoma, with adenocarcinoma, and with carcinoid. Sato *et al*[7] reported a case of small cell carcinoma with invasive squamous cell carcinoma. Sasajima *et al*[8] demonstrated one case of esophageal carcinoma showing multiple differentiations into oat

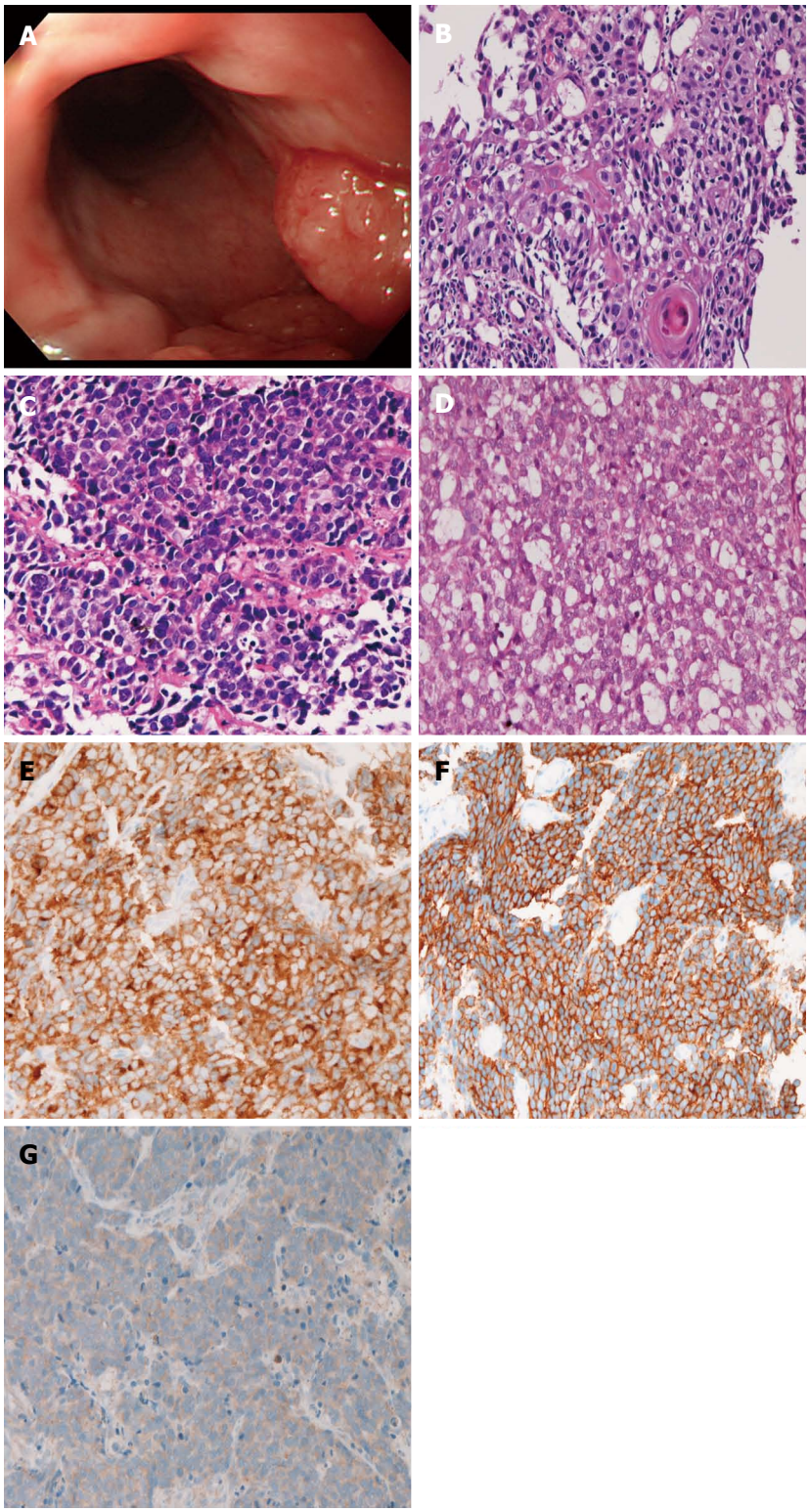

Figure 2 Case 2. A: Endoscopy. An elevated tumor is seen in the esophagus; B: Histology of the squamous cell carcinoma element of the esophageal tumor. A cancer pearl is seen [hematoxylin and eosin (HE), x 200]; C: Small cell carcinoma element of the esophageal carcinoma. The tumor cells show characteristic morphologies of small cell carcinoma (HE, x 200); D: Adenocarcinomatous element shows focal tubular formations (HE, x 200); E: CD56 is expressed in the small cell carcinoma component (x 200); F: KIT is expressed in the small cell carcinoma component (x 200); G: Platelet-derived growth factor-α is expressed in the small cell carcinoma component (x 200).

cell carcinoma, adenoid cystic carcinoma, adenocarcinoma, and squamous cell carcinoma. Mori *et al*[9] reported that 7 squamous cell foci and 2 adenocarcinoma foci were recognized in 10 small cell carcinomas. Attar *et al*[10] showed concomitant squamous cell carcinoma in small cell carcinoma. Beyer *et al*[11] mentioned that there was considerable histological heterogeneity in small cell carcinoma. Fujiwara *et al*[13] reported a case of small cell carcinoma with concomitant squamous cell carcinoma. Takubo *et al*[14] found a combination of small cell carcinoma and squamous cell carcinoma in 11 of 21 cases, and a combination of small cell carcinoma and mucoepidermid carcinoma in 1 of 21 cases. Medgyesy *et al*[15] found a combination of small cell carcinoma and adenocarcinoma in 1 of 8 cases, and a combination of small cell carcinoma and squamous cell carcinoma in 1 of 8 cases. Cho *et al*[16] identified a combination of basaloid squamous cell carcinoma and squamous cell carcinoma in 8 of 18 cases, a

combination of basaloid squamous cell carcinoma and adenocarcinoma in 3 of 18 cases, a combination of basaloid squamous cell carcinoma and small cell carcinoma in 2 of 18 cases. Uğraş *et al*[18] reported a combined carcinoma composed of small cell carcinoma and squamous cell carcinoma. Ishihara *et al*[19] found an esophageal combined carcinoma consisting of Pagetoid squamous cell carcinoma, choriocarcinoma, and mucoepidermoid carcinoma. Yamamoto *et al*[20] reported *in situ* and invasive squamous cell carcinomas were present in 3 of 6 cases of small cell carcinoma. Wu *et al*[21] reported that small cell carcinoma with squamous cell carcinoma was found in 3 of 9 cases. Yun *et al*[22] identified squamous differentiation in small cell carcinoma in 2 of 21 cases. Bilbeau *et al*[23] reported a case of small cell carcinoma with adenocarcinoma in a Barrett's esophagus. Maru *et al*[24] reported that a combination of small cell carcinoma and adenocarcinoma was seen in 15 of 40 cases, and a combination of small cell carcinoma and squamous cell carcinoma in 1 of 40 cases. Therefore, this literature review showed that combined carcinoma of the esophagus is not so rare among small cell esophageal carcinomas, and that the majority of combined carcinoma is associated with small cell carcinoma. The review also confirmed that esophageal combined carcinoma composed of small cell carcinoma and squamous cell carcinoma is the most common, followed by a combination of small cell carcinoma and adenocarcinoma. The present 2 cases also are the common type of combined esophageal carcinoma.

As mentioned above, small cell carcinoma is diagnosed by HE staining[31]. About 90% of small cell carcinoma has neuroendocrine features[31]. The neuroendocrine features can be demonstrated by immunohistochemical demonstration of neuroendocrine antigens such as chromogranin, synaptophysin, CD56, and neuron-specific enolase or by ultrastructural demonstration of neuroendocrine secretory vesicles[32]. Yamamoto *et al*[20] described that CD56, neuron-specific enolase, and chromogranin were positive in a small cell carcinoma component while they were negative in the squamous cell carcinoma component in 3 cases of combined esophageal carcinoma. They also demonstrated that both components were positive for cytokeratins and epithelial membrane antigen. Wu *et al*[21] described that esophageal small cell carcinomas were positive for neuron-specific enolase, chromogranin A, and synaptophysin in all 9 cases investigated. Yun *et al*[22] described that the percentage of endocrine markers in 21 esophageal small cell carcinomas was as follows: synaptophysin, 95%; CD56, 76%; chromogranin A, 62%, neuron-specific enolase, 62%, TTF-1, 71%; epithelial membrane antigen, 62%; cytokeratins, 57%; S100 protein, 19%. Maru *et al*[24] described that chromogranin was positive in 31 of 40 and synaptophysin in all 40 esophageal neoroendocrine carcinomas. In the present case, synaptophysin, CD56 and chromogranin were positive in the small cell carcinoma component in case 1, and CD56 and synaptophysin were positive in the small cell carcinoma component in case 2. In both cases in the present study, all the elements were positive for cytokeratin and epithelial membrane antigen. The non-small cell carcinoma components were negative for the neuroendocrine carcinoma. These findings are compatible with those of previous studies.

The present study has new findings: it showed positive expression of *KIT* and *PDGFRA* in the small cell carcinoma element of the 2 combined esophageal carcinomas. The present study also revealed that the squamous cell carcinoma and adenocarcinoma components were negative for KIT and PDGFRA protein and were negative for *KIT* and *PDGFRA* mutations in the esophageal combined carcinoma. KIT and PDGFRA are transmembranous receptor tyrosine kinase oncoproteins involved in carcinogenesis[33-35]. The vast majority of small cell carcinoma develops in the lung. In small cell lung carcinoma, KIT is frequently expressed, but no mutations of *KIT* gene have been recognized[36-46]. In small cell lung carcinoma, protein expression and mutations of PDGFRA are unknown. In extrapulmonary small cell carcinoma, KIT and PDGFRA proteins are frequently expressed, but there have been no mutations of *KIT* and *PDGFRA* genes found[46-48]. Many more studies of the *KIT* and *PDGFRA* gene status in esophageal combined carcinomas are necessary to elucidate the molecular mechanism of the carcinogenesis.

The biological behavior of these combined carcinomas of the esophagus is not known. However, it is thought that these combined carcinomas behave like small cell carcinoma, because the great majority of these combined carcinomas contain a small cell carcinoma element[1-24]. The option for treatment is not surgery but chemotherapy and radiation as in pulmonary small cell carcinoma[1-24]. The chemotherapy employed was cisplatin and etoposide[1-24]. Adjuvant radiation therapy may be effective. The combined carcinomas of the esophagus have a higher propensity for systemic metastases[1-24]. The survival rate is not clear because of a limited number of cases. However, survival was thought to be similar to that of pulmonary small cell carcinoma[1-24].

In summary, the authors presented 2 rare cases of esophageal combined carcinoma with double (squamous cell carcinoma and small cell carcinoma) and triplicate differentiation (squamous cell carcinoma, small cell carcinoma, and adenocarcinoma). The authors speculates that the combined carcinomas are basically small cell carcinomas with squamous and/or adenocarcinomatous differentiation. The present esophageal combined carcinomas may arise from enterochromaffin or totipotent stem cell of the esophagus. It is also possible that each element of the esophageal combined carcinomas may be derived from transdifferentiation of other elements. There were expressions of KIT and PDGFRA in the small cell carcinoma component of the esophageal combined carcinomas, but were negative for mutations of *KIT* and *PDGFRA*.

REFERENCES

1 **Rosen Y,** Mon S, Kim B. Small cell epidermoid carcinoma of the esophagus: An oat cell carcinoma. *Cancer* 1975; **36**: 1042-1049
2 **Ho KJ,** Herrera GA, Jones JM, Alexander CB. Small cell carcinoma of the esophagus: evidence for a unified histogenesis. *Hum Pathol* 1984; **15**: 460-468
3 **Reid HA,** Richrdson WW, Corrin B. Oat cell carcinoma of the esophagus. *Cancer* 1980; **45**: 2342-2347
4 **Reyes CV**, Chejfec G, Jao W, Gould VE. Neuroendocrine carcinomas of the esophagus. *Ultrastruct Pathol* 1980; **1**: 367-376
5 **Sarma DP**. Oat cell carcinoma of the esophagus. *J Surg Oncol* 1982; **19**: 145-150
6 **Doherty MA,** McIntyre M, Arnott SJ. Oat cell carcinoma of the esophagus: a report of six British patients with a review of the literature. *Int J Radiat Oncol Biol Phys* 1984; **10**: 147-152
7 **Sato T,** Mukai M, Ando N, Tashiro Y, Iri H, Abe O, Watanabe Y. Small cell carcinoma (non-oar cell type) of the esophagus concomitant with invasive squamous cell carcinoma and carcinoma in situ: A case report. *Cancer* 1986; **15**: 328-332
8 **Sasajima K**, Hayashi N, Yamashita K, Onda M, Takubo K. Oat cell carcinoma of the esophagus with multiple differentiation. *J Clin Gastroenterol* 1988; **10**: 667-671
9 **Mori M,** Matsukuma A, Adachi Y, Miyagahara T, Matsuda H, Kuwano H, Sugimachi K, Enjoji M. Small cell carcinoma of the esophagus. *Cancer* 1989; **63**: 564-573
10 **Attar BM**, Levendoglu H, Rhee H. Small cell carcinoma of the esophagus. Report of three cases and review of the literature. *Dig Dis Sci* 1990; **35**: 145-152
11 **Beyer KL**, Marshall JB, Diaz-Arias AA, Loy TS. Primary small-cell carcinoma of the esophagus. Report of 11 cases and review of the literature. *J Clin Gastroenterol* 1991; **13**: 135-141
12 **Law SY,** Fok M, Lam KY, Loke SL, Ma LT, Wong J. Small cell carcinoma of the esophagus. *Cancer* 1994; **73**: 2894-2899
13 **Fujiwara Y**, Nakagawa K, Tanaka T, Utsunomiya J, Nishigami T, Uematsu K. Small cell carcinoma of the esophagus combined with superficial esophageal cancer. *Hepatogastroenterology* 1996; **43**: 1360-1369
14 **Takubo K,** Nakamura K, Sawabe M, Arai T, Esaki Y, Miyashita M, Mafune K, Tanaka Y, Sasajima K. Primary undifferentiated small cell carcinoma of the esophagus. *Hum Pathol* 1999; **30**: 216-221
15 **Medgyesy CD,** Wolff RA, Putnam JB Jr, Ajani JA. Small cell carcinoma of the esophagus: the University of Texas M.D. Anderson Cancer Center experience and literature review. *Cancer* 2000; **88**: 262-267
16 **Cho KJ**, Jang JJ, Lee SS, Zo JI. Basaloid squamous carcinoma of the oesophagus: a distinct neoplasm with multipotential differentiation. *Histopathology* 2000; **36**: 331-340
17 **Bennouna J,** Bardet E, Deguiral P, Douillard JY. Small cell carcinoma of the esophagus: analysis of 10 cases and review of the published data. *Am J Clin Oncol* 2000; **23**: 455-459
18 **Uğraş S**, Akpolat N, Er M, Yalçýnkaya I, Karaayvaz M. Primary composite tumour with bipartite differentiation of the esophagus. *Acta Chir Belg* 2000; **100**: 39-43
19 **Ishihara A**, Mori T, Koono M. Diffuse pagetoid squamous cell carcinoma of the esophagus combined with choriocarcinoma and mucoepidermoid carcinoma: an autopsy case report. *Pathol Int* 2002; **52**: 147-152
20 **Yamamoto J**, Ohshima K, Ikeda S, Iwashita A, Kikuchi M. Primary esophageal small cell carcinoma with concomitant invasive squamous cell carcinoma or carcinoma in situ. *Hum Pathol* 2003; **34**: 1108-1115
21 **Wu Z**, Ma JY, Yang JJ, Zhao YF, Zhang SF. Primary small cell carcinoma of esophagus: report of 9 cases and review of literature. *World J Gastroenterol* 2004; **10**: 3680-3682
22 **Yun JP**, Zhang MF, Hou JH, Tian QH, Fu J, Liang XM, Wu QL, Rong TH. Primary small cell carcinoma of the esophagus: clinicopathological and immunohistochemical features of 21 cases. *BMC Cancer* 2007; **7**: 38
23 **Bibeau F**, Chateau MC, Guiu M, Assenat E, Azria D, Lavaill R, Ychou M, Boissière-Michot F. Small cell carcinoma with concomitant adenocarcinoma arising in a Barrett's oesophagus: report of a case with a favourable behaviour. *Virchows Arch* 2008; **452**: 103-107
24 **Maru DM**, Khurana H, Rashid A, Correa AM, Anandasabapathy S, Krishnan S, Komaki R, Ajani JA, Swisher SG, Hofstetter WL. Retrospective study of clinicopathologic features and prognosis of high-grade neuroendocrine carcinoma of the esophagus. *Am J Surg Pathol* 2008; **32**: 1404-1411
25 **Terada T**, Kawaguchi M, Furukawa K, Sekido Y, Osamura Y. Minute mixed ductal-endocrine carcinoma of the pancreas with predominant intraductal growth. *Pathol Int* 2002; **52**: 740-746
26 **Terada T**, Kawaguchi M. Primary clear cell adenocarcinoma of the peritoneum. *Tohoku J Exp Med* 2005; **206**: 271-275
27 **Terada T.** Gastrointestinal stromal tumor of the uterus: a case report with genetic analyses of c-kit and PDGFRA genes. *Int J Gynecol Oncol* 2009; **28**: 29-34
28 **Terada T**. Primary multiple extragastrointestinal stromal tumors of the omentum with different mutations of c-kit gene. *World J Gastroenterol* 2008; **14**: 7256-7259
29 **Terada T**. Primary extragastrointestinal stromal tumor of the transverse mesocolon without c-kit mutations but with PDGFRA mutations. *Med Oncol* 2009; **26**: 233-237
30 **Terada T**. Mediastinal seminoma with multiple KIT gene mutations. *Pathology* 2009; **41**: 695-697
31 **Travis W,** Nicholson S, Hirsch FR et al. Small cell carcinoma. In: Travis WD, Blambillia E, Muller-Hermelink K, Harris CC, editors. WHO classification of tumour. Pathology and genetics of tumours of the lung pleura, thymus and heart. Lyon: IARC press, 2004: 31-34
32 **Miettinen M**, Lasota J. Gastrointestinal stromal tumors: review on morphology, molecular pathology, prognosis, and differential diagnosis. *Arch Pathol Lab Med* 2006; **130**: 1466-1478
33 **Hirota S**, Isozaki K, Moriyama Y, Hashimoto K, Nishida T, Ishiguro S, Kawano K, Hanada M, Kurata A, Takeda M, Muhammad Tunio G, Matsuzawa Y, Kanakura Y, Shinomura Y, Kitamura Y. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. *Science* 1998; **279**: 577-580
34 **Hirota S,** Ohashi A, Nishida T, Isozaki K, Kinoshita K, Shinomura Y, Kitamura Y. Gain-of-function mutations of platelet-derived growth factor receptor alpha gene in gastrointestinal stromal tumor. *Gastroenterol* 2003; **125**: 660-667
35 **Miettinen M,** Lasota J. KIT (CD117): a review on expression in normal and neoplastic tissue, and mutations and their clinicopathologic correlation. *Appl Immunohistochem Mol Morphol* 2005: **13**: 205-220
36 **LaPoint RJ,** Bourne PA, Wang HL, Xu H. Coexpression of c-kit and bcl-2 in small cell carcinoma and large cell neuroendocrine carcinoma of the lung. *Appl Immunohistochem Mol Morphol* 2007; **15**: 401-406
37 **López-Martin A**, Ballestín C, Garcia-Carbonero R, Castaño A, Lopez-Ríos F, López-Encuentra A, Sánchez-Cespedes M, Castellano D, Bartolomé A, Cortés-Funes H, Paz-Ares L. Prognostic value of KIT expression in small cell lung cancer. *Lung Cancer* 2007; **56**: 405-413
38 **Micke P**, Basrai M, Faldum A, Bittinger F, Rönnstrand L, Blaukat A, Beeh KM, Oesch F, Fischer B, Buhl R, Hengstler JG. Characterization of c-kit expression in small cell lung cancer: prognostic and therapeutic implications. *Clin Cancer Res* 2003; **9**: 188-194
39 **Camps C**, Sirera R, Bremnes RM, Garde J, Safont MJ, Blasco A, Berrocal A, Sánchez JJ, Calabuig C, Martorell M. Analysis of c-kit expression in small cell lung cancer: prevalence and prognostic implications. *Lung Cancer* 2006; **52**: 343-347
40 **Rossi G,** Cavazza A, Marchioni A, Migaldi M, Bavieri M,

Facciolongo N, Petruzzelli S, Longo L, Tamberi S, Crinò L. Kit expression in small cell carcinomas of the lung: effects of chemotherapy. *Mod Pathol* 2003; **16**: 1041-1047
41 **Naeem M**, Dahiya M, Clark JI, Creech SD, Alkan S. Analysis of c-kit protein expression in small-cell lung carcinoma and its implication for prognosis. *Hum Pathol* 2002; **33**: 1182-1187
42 **Mojica WD**, Saxena R, Starostik P, Cheney RT. CD117+ small cell lung cancer lacks the asp 816--> val point mutation in exon 17. *Histopathology* 2005; **47**: 517-522
43 **Boldrini L**, Ursino S, Gisfredi S, Faviana P, Donati V, Camacci T, Lucchi M, Mussi A, Basolo F, Pingitore R, Fontanini G. Expression and mutational status of c-kit in small-cell lung cancer: prognostic relevance. *Clin Cancer Res* 2004; **10**: 4101-4108
44 **Burger H,** Den Bakker MA, Stoter G, Verweij J, Nooter K. Lack of c-kit exon 11 activating mutations in c-kit/CD117-positive SCLC tumor specimens. *Eur J Cancer* 2003; **39**: 793-799
45 **Sihto H**, Sarlomo-Rikala M, Tynninen O, Tanner M, Andersson LC, Franssila K, Nupponen NN, Joensuu H. KIT and platelet-derived growth factor receptor alpha tyrosine kinase gene mutations and KIT amplifications in human solid tumors. *J Clin Oncol* 2005; **23**: 49-57
46 **Terada T**. Primary small cell carcinoma of the mediastinum: a case report with immunohistochemical and molecular genetic analyses of KIT and PDGFRA genes. *Med Oncol* 2009; **26**: 247-250
47 **Terada T**. Primary small cell carcinoma of the ureter: a case report involving immunohistochemical and molecular genetic analyses of KIT and PDGFRA genes. *Pathology* 2010; **42**: 101-102
48 **Terada T**. Autopsy case of primary small cell carcinoma of the urinary bladder: KIT and PDGFRA expression and mutations. *Pathol Int* 2009; **59**: 247-250

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CASE REPORT

Esophageal space-occupying lesion caused by Ascaris lumbricoides

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Abstract

Ascaris lumbricoides is the largest intestinal nematode parasite of man, which can lead to various complications because of its mobility. As the esophagus is not normal habitat of Ascaris, the report of esophageal ascariasis is rare. An old female presented with dysphagia after an intake of several red bean buns and haw jellies. The barium meal examination revealed a spherical defect in the lower esophagus. Esophageal bezoar or esophageal carcinoma was considered at the beginning. The patient fasted, and received fluid replacement treatment as well as some oral drugs such as proton pump inhibitor and sodium bicarbonate. Then upper gastrointestinal endoscopy was done to further confirm the diagnosis and found a live Ascaris lumbricoides in the gastric antrum and two in the duodenal bulb. The conclusive diagnosis was ascariasis. The esophageal space-occupying lesion might be the entangled worm bolus. Anthelmitnic treatment with mebendazole improved patient's clinical manifestations along with normalization of the radiological findings during a 2-wk follow-up. Authors report herein this rare case of Ascaris lumbricoides in the esophagus, emphasizing the importance of awareness of this parasitic infection as it often presents with different and unspecific symptoms.

Key words: Ascariasis; Ascaris lumbricoides; Esophagus; Endoscope; Radiograph

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INTRODUCTION

Ascaris lumbricoides is the giant intestinal roundworm, causing infection of the gastrointestinal tract and affects approximately one quarter of the world's population. In clinical observations, the majority of patients are infected with intestinal ascariasis. Adult roundworms can be stimulated to migrate to any orifice by stressful conditions such as gastrointestinal disease, fever, anesthesia and anthelmintic drugs, so some complications such as acute cholecystitis, acute cholangitis, and acute pancreatitis caused by ascariasis of bile or pancreatic ducts have been reported. However, demonstration of the worms in esophagus is extremely rare. A case of esophageal ascariasis in a 15-year-old boy was reported by an Indian author in 1999[1], but it did not discuss the possible mechanism. Herein, we describe a case that showed a space-occupying lesion of the esophagus in an old woman caused by Ascaris and discuss the likely causes.

CASE REPORT

A 70-year-old female presented with recurrent dysphagia for 4 years, and was admitted to our hospital with complaints of symptoms worsening in the past 3 d. The patient complained that the dysphagia often occurred when she ate too fast, which was unrelated to the position or season and relieved by inducing vomiting through physical stimulation. She also had abdominal distension and frequent belching. Three days before admission, the symptoms of dysphagia could not be relieved by inducing vomiting after an intake of several red bean buns and haw jellies. Barium meal examination in a local hospital revealed a spherical defect in the lower esophagus allowing little barium to pass through (Figure 1). She had no specific past medical history which included digestive system disease. Upon physical examination, her general condition was normal, temperature 37 ℃, blood pressure 120/75 mmHg, heart rate 64 beats/min, and respiratory rate of 18 breaths/min. She was thin but no signs of anemia. The abdomen was soft with normal bowel sounds and no peritoneal signs. The laboratory results were as follows: Hb 139 g/L, white blood cell 11.54 × 10^9/L, neutrophils 77.8%, eosinophils 0.3%. Markers for hepatitis A, B, C were negative. Kidney and liver function tests and serum electrolytes were normal. Fecal examination was not available due to absence of stools in the hospital. X-ray films of the chest and abdomen were performed immediately upon admission to our hospital and showed a globular high density shadow on the right side of trachea in the mediastinum, as well as a strand-like high density shadow in the right upper quadrant of abdomen, and excess barium remained in the colon, and lumbar spondylosis. We considered the following diagnoses: hiatal hernia, esophageal bezoar or esophageal carcinoma on the basis of the radiological appearance.

The patient fasted and was given fluid replacement treatment. Other oral drugs such as proton pump inhibitor (PPI) and sodium bicarbonate were also administered considering the possible existence of hiatal hernia and esophageal bezoar, while soybean oil was taken orally to help the barium pass with the feces. To further confirm the diagnosis, the patient underwent upper gastrointestinal endoscopy on the second day of admission. Endoscopy showed the mucous membrane of esophagus to be smooth and clear except for some slight congestion in the lower part; the body of stomach was normal and a live Ascaris lumbricoides worm was seen in the gastric antrum, where mucosa was thinner; there was a diverticula approximately 0.6 cm × 0.6 cm in size in the posterior wall of duodenal bulb and another two live worms were present (Figure 2). The congestion of duodenal mucosa also appeared. Endoscopic diagnoses were esophagitis, atrophic antral gastritis, duodenitis with diverticulum, and ascariasis.

The patient reported a history of passage of worms in stools, and a history of eating raw vegetables. She received mebendazole for anthelmitnic treatment along with PPI therapy. The patient improved symptomatically during a 2-wk follow-up, and did pass 5-6 worms with feces. Repeated barium examination for upper gastrointestinal tract was unremarkable (Figure 3).

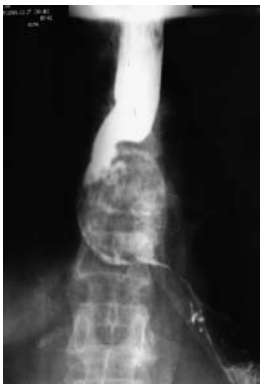

Figure 1 Barium meal examination revealed a spherical filling defect in the lower esophagus.

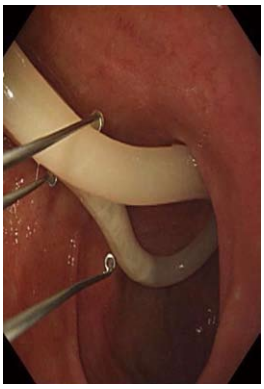

Figure 2 A live Ascaris worm appeared in the duodenal bulb, which was extracted with a grasper.

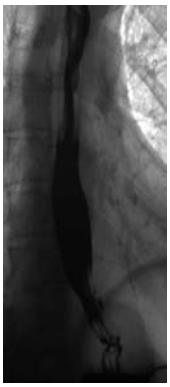

Figure 3 The defect filling disappeared in repeated barium examinations.

DISCUSSION

Ascaris lumbricoides, the largest intestinal nematode parasite of man, are commonly seen in rural areas of China, especially among people with poor hygienic conditions and/or having a habit of eating raw food. However, the prevalence of ascariasis has declined with improvement of sanitation and the application of pesticides and chemi-

cal fertilizers. The serious harm and complications of this round worm should be stressed. Ascaris larvae can cause transient eosinophilic pulmonary infiltrates (Loeffler's syndrome) when they migrate through the lungs, which is characterized by pulmonary infiltrates and peripheral blood eosinophilia. Infection of adult Ascaris lumbricoides, which mostly inhabit the jejunum and ileum, usually presents with anorexia, nausea and vomiting, intermittent periumbilical pain, and malnutrition. More serious complications of Ascaris infection may occur when a large worm burden is present in the lumen of the intestine, such as intestinal obstruction, intussusception, volvulus or even gangrene. Intestinal hemorrhage[2] and perforation by Ascaris have also been reported, with the latter being able to lead to acute diffuse peritonitis or peritoneal granuloma[3]. In addition, Ascaris infection can cause allergic reactions, presenting with urticaria, skin itch, angioneurotic edema or even eosinophilic cholecystitis[4].

When the living environment becomes unfavorable such as gastrointestinal disease, hunger, fever, failed deworming therapy or impaction of a mass of worms in the intestinal lumen, adult Ascaris will try to enter into any orifice and advance into any channel leading off from it. Then various complications are encountered. The worms commonly enter the biliary or pancreatic ducts, causing cholecystitis, cholangitis, liver abscess, and pancreatitis. Ascaris may migrate into appendix as well, resulting in appendiceal colic and appendicitis. Ascaris has also been found in the lacrimal passage by being regurgitated into the nasolacrimal duct when they accidentally enter nasopharynx; in the air way causing mechanical asphyxia; and in the urethra and urinary bladder through vesico-intestinal fistulae or transanal migration causing urinary retention. Moreover, the emergence of an Ascaris from mouth, nostrils and external auditory meatus has been documented. Esophagus ascariasis is extremely rare, because the esophagus is not normal habitat of Ascaris as it prefers an alkaline environment and rarely travels from the jejunum and duodenum to the stomach (an acid environment) and then to esophagus.

The patient in our case is at high risk of ascariasis as she is from rural area and in favor of eating fresh vegetables. This old woman was admitted to the hospital due to dysphagia with an abnormal barium study of the esophagus. In Figure 1, we can find several high-density string shadows and globular shadows in the esophageal spherical filling defect, which actually are Ascaris worms. The most likely explanation in our case is that the worms were forced to migrate by gastroduodenal antiperistalsis, which is induced by eating too many red bean buns and haw jellies. Subsequently these worms became entangled with each other to form a small worm bolus in the alkaline esophagus, resulting in the symptom of dysphagia. The patient received treatment of soybean oil after admission, and she drank lidocaine hydrochloride mucilage before endoscopic examination, which relaxed the lower esophageal sphincter, possibly stimulating the worms. All of these measures induced the migration of the Ascaris bolus to the gastrointestinal tract. Therefore, the esophagus appeared normal except for some slight mucosal congestion on endoscopy. After anthelmintic therapy, more ascarides were passed out with feces, and a repeated barium study of upper gastrointestinal showed that the former defect filling had disappeared, which confirmed that the abnormal radiological appearance in the esophagus was a worm bolus. In a previous case[1] with similar radiographic appearance in the esophagus, the boy later vomited out six live Ascaris worms, and his esophageal lumen and mucosa were normal on the barium examination done the next day. Because of lack of exact causative factors in this case, we consider that children's anatomically smaller intestine and larger worm loads caused the rapid transit of worms across the esophagus. This kind of anti-peristaltic migration of Ascaris is truly uncommon.

As our patient had a history of recurrent dysphagia and eating too many haw jellies before admission, we highly suspected that hiatal hernia combining with esophageal bezoar might cause this kind of space-occupying lesion at the beginning. However, bezoars rarely form in the esophagus and are often associated with structural and functional abnormalities of the esophagus, such as achalasia and hiatal hernia. Esophageal bezoar is also a complication of enteral feeding and the predisposing factors include mechanical ventilation, supine position, neurological diseases, diabetes mellitus, hypothyroidism, obesity and history of partial gastrectomy[5]. Therefore, we performed the gastrointestinal endoscopy to clarify the diagnosis. The unexpected result suggests that careful history-taking and complete examinations are necessary.

In conclusion, we reported a case of an old woman with esophageal ascariasis and discussed the possible causes. We often lack of awareness of Ascaris infection because of the dramatically decreasing incidence and several different and unspecific symptoms of this infection. This report also listed some other common and rare complications, and we would like to warn all medical workers that we should pay more attention to such a disease and reduce any misdiagnosis in the future work.

REFERENCES

1 **Gandhi D**, Srivastava DN, Batra B, Chandra S. Radiological demonstration of ascaris in esophagus. *Indian Pediatr* 1999; **36**: 1270-1272

2 **Suzuki A**, Yabushita Y, Takahashi H, Inamori M, Nakao S, Suzuki K, Iida H, Endo H, Akiyama T, Ikeda T, Sakamoto Y, Fujita K, Yoneda M, Goto A, Abe Y, Kirikoshi H, Kobayashi N, Kubota K, Saito S, Nakajima A. Education and imaging. Gastrointestinal: ascariasis. *J Gastroenterol Hepatol* 2008; **23**: 1770

3 **Chen D**, Bo L. Ascaris peritonitis and peritoneal granuloma in China. *J Gastroenterol* 1997; **32**: 826-829

4 **Kaji K**, Yoshiji H, Yoshikawa M, Yamazaki M, Ikenaka Y, Noguchi R, Sawai M, Ishikawa M, Mashitani T, Kitade M, Kawaratani H, Uemura M, Yamao J, Fujimoto M, Mitoro A, Toyohara M, Yoshida M, Fukui H. Eosinophilic cholecystitis along with pericarditis caused by Ascaris lumbricoides: a case report. *World J Gastroenterol* 2007; **13**: 3760-3762

5 **Marcus EL**, Arnon R, Sheynkman A, Caine YG, Lysy J. Esophageal obstruction due to enteral feed bezoar: A case report and literature review. *World J Gastrointest Endosc* 2010; **2**: 352-356

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January 20-21, 2012
American Gastroenterological Association Clinical Congress of Gastroenterology and Hepatology
Miami Beach, FL 33141, United States

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The Future of Obesity Treatment
London, United Kingdom

February 16-17, 2012
4th United Kingdom Swallowing Research Group Conference
London, United Kingdom

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Management of Barretts Oesophagus: Everything you need to know
Cambridge, United Kingdom

February 24-27, 2012
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Montreal, Canada

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International Conference on Nutrition and Growth 2012
Paris, France

March 7-10, 2012
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Omaha, NE 68197, United States

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Orlando, FL 32808, United States

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26th Annual New Treatments in Chronic Liver Disease
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San Antonio, TX 78249, United States

March 31-April 1, 2012
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Milwaukee, WI 53202, United States

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Singapore, Singapore

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Prague, Czech

April 18-20, 2012
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San Diego, CA 92101, United States

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Como, Italy

September 6-8, 2012
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Bologna, Italy

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The Viral Hepatitis Congress
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September 8-9, 2012
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November 3-4, 2012
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Dnepropetrovsk, Ukraine

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The Liver Meeting
San Francisco, CA 94101, United States

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World Journal of Gastroenterology (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a weekly, open-access (OA), peer-reviewed journal supported by an editorial board of 1352 experts in gastroenterology and hepatology from 64 countries.

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The role of academic journals is to exhibit the scientific levels of a country, a university, a center, a department, and even a scientist, and build an important bridge for communication between scientists and the public. As we all know, the significance of the publication of scientific articles lies not only in disseminating and communicating innovative scientific achievements and academic views, as well as promoting the application of scientific achievements, but also in formally recognizing the "priority" and "copyright" of innovative achievements published, as well as evaluating research performance and academic levels. So, to realize these desired attributes of *WJG* and create a well-recognized journal, the following four types of personal benefits should be maximized. The maximization of personal benefits refers to the pursuit of the maximum personal benefits in a well-considered optimal manner without violation of the laws, ethical rules and the benefits of others. (1) Maximization of the benefits of editorial board members: The primary task of editorial board members is to give a peer review of an unpublished scientific article via online office system to evaluate its innovativeness, scientific and practical values and determine whether it should be published or not. During peer review, editorial board members can also obtain cutting-edge information in that field at first hand. As leaders in their field, they have priority to be invited to write articles and publish commentary articles. We will put peer reviewers' names and affiliations along with the article they reviewed in the journal to acknowledge their contribution; (2) Maximization of the benefits of authors: Since *WJG* is an open-access journal, readers around the world can immediately download and read, free of charge, high-quality, peer-reviewed articles from *WJG* official website, thereby realizing the goals and significance of the communication between authors and peers as well as public reading; (3) Maximization of the benefits of readers: Readers can read or use, free of charge, high-quality peer-reviewed articles without any limits, and cite the arguments, viewpoints, concepts, theories, methods, results, conclusion or facts and data of pertinent literature so as to validate the innovativeness, scientific and practical values of their own research achievements, thus ensuring that their articles have novel arguments or viewpoints, solid evidence and correct conclusion; and (4) Maximization of the benefits of employees: It is an iron law that a first-class journal is unable to exist without first-class editors, and only first-class editors can create a first-class academic journal. We insist on strengthening our team cultivation and construction so that every employee, in an open, fair and transparent environment, could contribute their wisdom to edit and publish high-quality articles, thereby realizing the maximization of the personal benefits of editorial board members, authors and readers, and yielding the greatest social and economic benefits.

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6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

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Books

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15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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