CASE STUDY

Hodgkin and Reed-Sternberg cells in bone marrow aspirations of a patient with advanced classical Hodgkin lymphoma

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ABSTRACT

Classical Hodgkin lymphoma (CHL) is a unique type of lymphoma because of the extraordinary and unexplained scarcity of its neoplastic Hodgkin Reed-Sternberg (HRS) cells that derived from clonal germinal center B cells with rearranged immunoglobulin genes bearing crippling mutations. The occurrence of these cells in the bone marrow aspirations are considered rare. Their presence is an expression of widely disseminated disease and it indicates poor prognosis. We report a case of a 24-year-old female with relapsed Hodgkin lymphoma, after eleven years in remission with the standard chemotherapy regime. She was initially diagnosed during childhood with CHL stage IIIB. On this current presentation, she was noted to have cervical lymphadenopathy during her antenatal check-up. The lymph nodes biopsy confirmed relapse of the disease; however, there was no evidence of bone marrow infiltration. She was given various chemotherapy regimes in which she was refractory to. At this point, repeated bone marrow aspiration interestingly revealed the presence of HRS cells. The immunophenotyping analysis by flow cytometry revealed a small population of cells expressing CD20, CD15, and CD30 that further supported the presence of HRS cells in the bone marrow aspirate. The bone marrow biopsy confirmed infiltration of scattered Reed-Sternberg cells and mononuclear Hodgkin cells in a reactive background. In conclusion, we highlight the presence of the rare HRS cells in this patient with advanced relapsed Hodgkin lymphoma.
Keywords: Hodgkin Reed-Sternberg cells, classical Hodgkin lymphoma, bone marrow aspirates, flow cytometry.

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INTRODUCTION

Hodgkin Lymphoma (HL) is a group of lymphomas of mainly nodal origin with similar clinical and histological features (1). HL encompasses 95% of classical Hodgkin lymphomas (CHL) and the rest are of nodular lymphocyte predominant Hodgkin lymphoma (NLPHL). These two entities differ in their aetiology, clinical features, histopathological features, immunophenotype and molecular genetic features. NLPHL is an uncommon monoclonal B cell neoplasm characterized by large neoplastic cells known as popcorn or lymphocyte predominant cells (LP cells) in an inflammatory background. NLPHL is considered to be of germinal centre B-cell origin. It is managed differently from classical HL. The prognosis is good; however, late relapses and transformation to high grade non-Hodgkin lymphoma can occur (1,2).

Classical Hodgkin lymphoma (CHL) is a monoclonal lymphoid neoplasm representing less than 1% of all *de novo* neoplasms occurring each year worldwide (2). It is unique among lymphomas due to the distinctive malignant multinucleated Hodgkin Reed-Sternberg (HRS) cells as well as the mononuclear Hodgkin cells (2,3). Generally, there are four subtypes of CHL; nodular sclerosis, mixed cellularity, lymphocyte-rich, and lymphocyte-depleted. These subtypes may differ in the clinicopathological aspects, however, the immunophenotype of the tumor cells remain the same. The distinctive Reed-Sternberg and Hodgkin cells are nearly always CD30 positive. Most of the CHL cases are also CD15 positive whilst CD20 is positive with varied intensity in 30-40% of cases. Hodgkin cells and Reed-Sternberg (HRS) cells are derived from the same clonal B cells population. The rearranged immunoglobulin (Ig) genes of the tumor cells harbor a high load of somatic hypermutations in the variable region of the Ig heavy chain genes usually without signs of ongoing mutations. These clonal rearrangements are usually detectable only in the DNA of isolated single HRS cells and not in whole tissue DNA (2).

Hodgkin lymphoma (HL) is a curable disease in adults. With modern treatment strategies majority of the HL patients with various anatomical stages and histological subtypes can be cured (4). Improvement in its diagnosis and staging, treatment of the disease, as well as its supportive management, has contributed in the outcome of the disease (5). Nevertheless, relapse or progression of the disease after the initial treatment still occurs in 20-30% of the patients. The pathogenesis and mechanism of treatment failure are still not well understood partly due to the difficulties in studying the molecular phenotype of the rare malignant HRS cells (3).

We report a case of advanced CHL with presence of Reed-Sternberg cells in the bone marrow where the diagnosis of CHL stage IIIB was made during childhood period. She was treated successively with chemotherapy. However, the disease relapsed and these cells were demonstrated in her bone marrow samples only after the disease had disseminated and became refractory to chemotherapy.

CASE REPORT

A 24-year-old Chinese female was first diagnosed with classical Hodgkin lymphoma at the age of 11 years. She was treated with the standard ABVD regime (Adriamycin, bleomycin, vinblastine, dacarbazine) and achieved remission for 11 years until she presented to us again with a mass in the neck during pregnancy. One month post-partum, a biopsy from the cervical lymph node was performed to reveal relapsed CHL, mixed cellularity subtype. The computed tomography (CT) scan demonstrated extensive cervical, mediastinal, axillary, abdominal, and inguinal lymphadenopathy. At this point of time, bone marrow examination showed no evidence of lymphomatous infiltration. The patient was treated with five cycles of BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone) chemotherapy. Mid-cycle assessment with PET scan 5 months later showed evidence of active lymphoma in the chest node and bone involvement. In view of these findings, the chemotherapy regimen was changed to ICE (ifosfamide, carboplatin, etoposide) and the patient was planned for autologous stem cell transplantation (ASCT). Following completing two cycles of ICE, stem cells harvesting was attempted twice but unfortunately failed. In view of disease progression, as evidenced by CT scan findings that demonstrated worsening of the mediastinal, intra-abdominal lymphadenopathy with lung, spleen, liver and urinary bladder infiltration, chemotherapy regimen was escalated to modified IGEV (ifosfamide, gemcitabine, vinorelbine).

Half way through this chemotherapy regime, her full blood count exhibited mild anemia, mild leukocytosis, and severe thrombocytopenia (haemoglobin: 111.0 g/L; MCV: 87.6 fl; MCH: 30.1 pg; white cells count: 10.1x 10^9/L; platelet count: 4 x 10^9/L). The full blood picture showed occasional nucleated red cells and a few atypical lymphocytes but no abnormal lymphoid cells. There was no leukoerythroblastic picture noted. However, the bone marrow aspirates demonstrated a normocellular marrow but there was an occasional presence of very large cells. These cells exhibited binuclearity with inclusion-like nucleoli and abundant cytoplasm resembling the typical Reed-Sternberg (RS) cells (Figure 1 A & B). There was also the presence of mononuclear Hodgkin cells, which are large cells of a similar appearance but with a single nucleus. Interestingly, the immunophenotyping analysis demonstrated a small abnormal population (2.39%) gated at the CD19 positive area which showed positivity for CD45 (dim), CD20 (dim), CD15, and CD30. This small population consisted of large cells with complex characteristics as evidenced from the forward and side scatter gating (Figure 2). The bone marrow biopsy showed infiltration by scattered Reed-Sternberg cells and mononuclear Hodgkin cells in reactive background consisting of small lymphocytes, epithelioid histiocytes, and occasional neutrophils and eosinophils (Figure 3). The immunohistochemistry staining results showed these cells to be CD30+ (strong and diffuse); CD15 and PAX5 were positive in a few of these cells. However, they were negative for CD20. The reticulin stain exhibited coarse fibre network. These findings were concluded as bone marrow infiltration by classical Hodgkin lymphoma.

Her condition took a downfall when she developed sepsis secondary to the infected chemoport site, thus chemotherapy was delayed. Furthermore, her serum alkaline phosphatase and bilirubin levels were increasing in trend. Urgent ultrasound revealed hepatosplenomegaly and multiple ill-defined hypoechoic lesions within the spleen, which may represent multiple abscesses or lymphomatous infiltration. Later, she developed acute renal failure, which resulted in alteration in her level of consciousness and a few episodes of seizures. Owing to the clinical features coupled with the full blood picture findings of microangiopathic haemolytic anaemia (MAHA), she was treated for thrombotic thrombocytopenic purpura (TTP) and underwent three cycles of plasmapheresis. A CT brain scan was done and demonstrated left frontal infarct with haemorrhagic transformation as well as marked perilesional oedema and mass effect. However, lymphatic tumoral infiltration could not be excluded. Eventually, she developed multiorgan failure and succumbed to her condition.

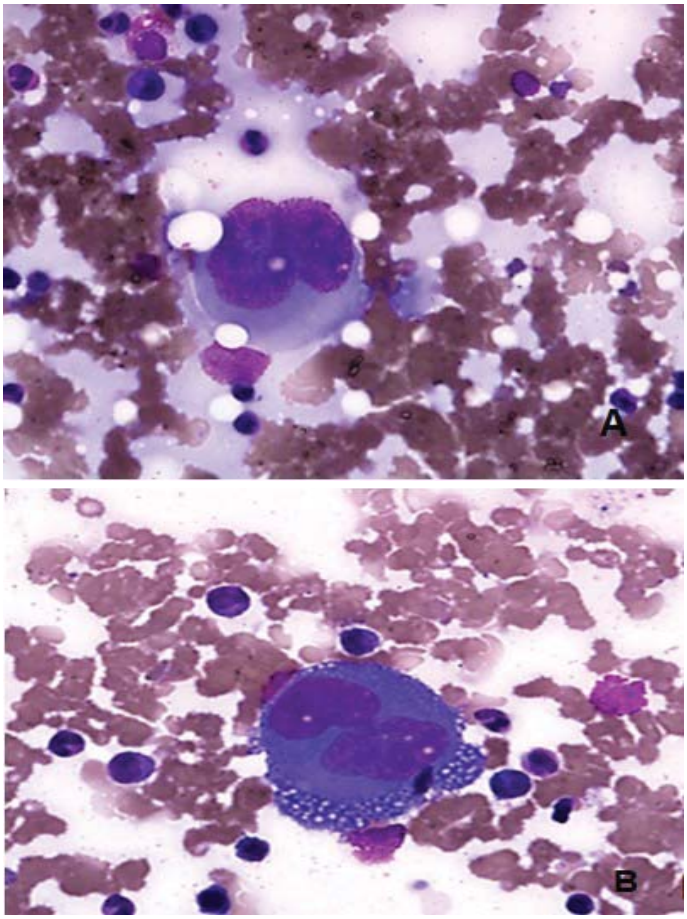

Figure 1. Bone marrow aspirates (A & B) showing Reed-Sternberg cells- very large cells that exhibit paired nuclei with inclusion-like nucleoli and abundant cytoplasm. MGG x600.

DISCUSSION

Neoplastic cells, either the classical Reed-Sternberg (RS) cells or the Hodgkin cells characteristically represent only a minority of the cellular infiltrate with an incidence ranging from 0.1- 10% (2). Nevertheless, the confirmation of HL requires morphologic diagnosis of the neoplastic cells with the appropriate cellular background along with the result of immunophenotyping as cells resembling Reed-Sternberg cells can be found in cases of B and T lymphomas, melanomas, sarcoma, and in some reactive conditions such as infectious mononucleosis, which are common in populations across the globe (6).

In 1998, Küppers *et al*. performed molecular studies in a single cell of HRS cells in HL (7). Their study showed that HRS cells in CHL, as well as NLPHL, originate from the germinal center (GC) B cells in most cases, if not all. HRS cells in NLPHL represents transformed antigen-selected GC B cells with evidence of ongoing immunoglobulin (Ig) V gene mutation. Whereas HRS cells in CHL appear to often or always derive from GC B cells that have lost the capacity to express a functional antigen receptor. Piccaluga *et al*. (6) also mentioned that HRS cells are sustained by an autocrine and/or paracrine production of several cytokines, including IL-5, IL-8, IL-9, CCL- 5, and CCL-28. The release of these molecules is also accountable for most of the symptoms observed in patients with HL, in addition to the ability of the neoplastic cells to escape from growth controls and immunosurveillance.

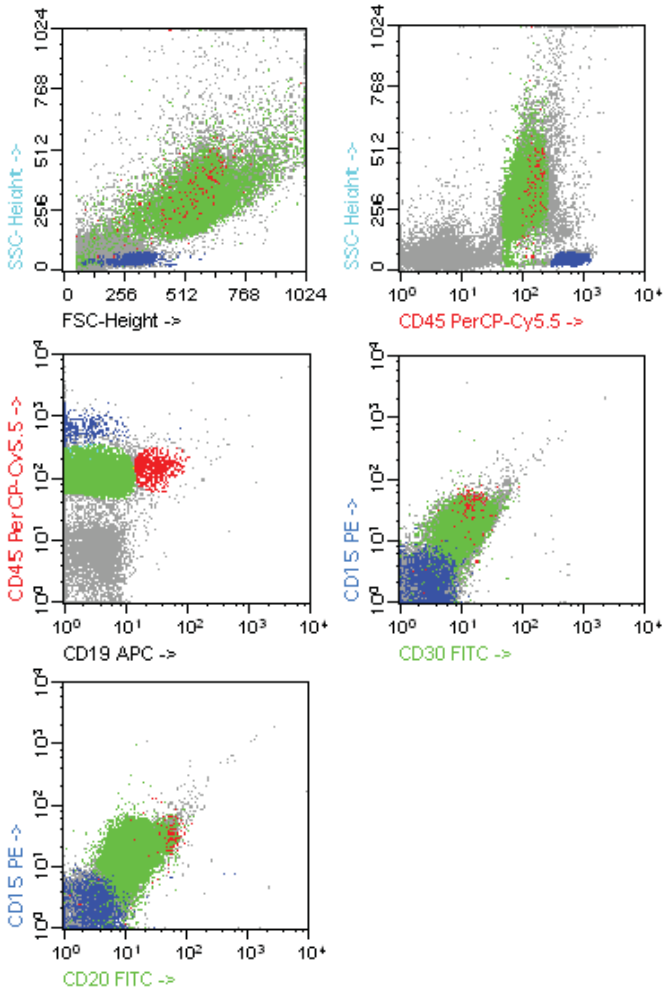

Figure 2. Flow cytometry immunophenotyping analysis showing the small (2.39%) abnormal populations (highlight-red). These cells expressed CD45 (dim), CD19, CD20, CD15, and CD30.

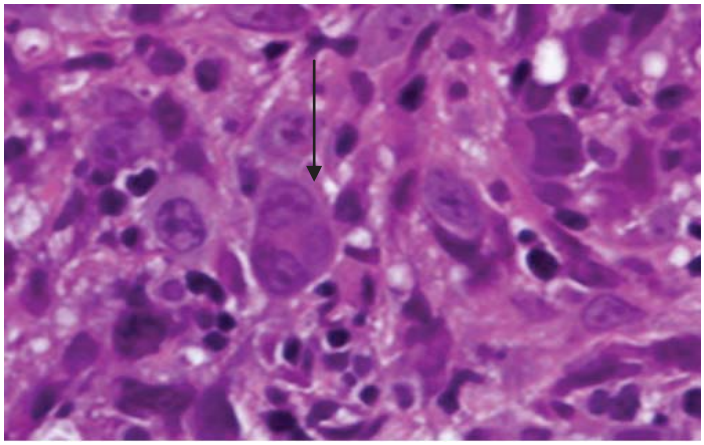

Figure 3. Bone marrow biopsy section showing focally hypercellular marrow space displaying infiltration by scattered Hodgkin cells and Reed-Sternberg cells (arrow). H&E x 600.

Bone marrow involvement is rare in patients with HL. Its incidence varies between 4% and 14% in the series reported during the past 20 years (8). As the bone marrow lacks lymphatics, infiltration of the bone marrow by Hodgkin's lymphoma indicates vascular dissemination of the disease (stage IV). The incidence of bone marrow involvement in Hodgkin's lymphoma varies with the histologic subtype: 10% in classical Hodgkin's mixed cellularity, approximately 1% in lymphocyte predominant and lymphocyte rich CHL, and 3% in nodular sclerosis subtype (9). The reason for this rarity can be due to the scattered focal lesions which may not be aspirated by the bone marrow aspirate needle, and also in HL the bone marrow tends to have fibrous tissue, making their aspiration more difficult (10). Fibrosis is a common finding in Hodgkin's lesions in the bone marrow and is not limited to nodular sclerosis or lymphocyte depletion variants (9). RS cells are found mainly in bone marrow of patients with generalized advanced stages of HL (10). Thus, the presence of RS cells in bone marrow is an expression of widely disseminating disease (11). Therefore, features of diffuse fibrosis associated with polyploid RS cell variants or large abnormal mononuclear cells with huge nucleoli are sufficient evidence for the determination of marrow involvement on random biopsy (11).

Morphologic findings and immunohistochemical stains are vital in the diagnosis of CHL (12). In the majority of cases, flow cytometry analysis is of little or no value to the detection of HRS and the diagnosis of CHL, as neoplastic cells are rarely seen in the cytological preparations (1), and it usefulness is limited to tissue samples such as lymph nodes (12). Recently, Fromm and Wood (13) described a method of identifying HRS cells in lymph nodes by flow cytometry using a single-tube (6-colour assay) with high sensitivity and specificity. Also, they proposed that this method might obviate the need for immunohistochemistry in many cases. Furthermore flow cytometry offered several potential benefits in the diagnosis of CHL in which it is more sensitive in equivocal or possibly negative cases by morphology. It has a rapid turnaround time with significant cost effectiveness compared to the elaborate immunohistochemical panels. In our present case we have concluded that there was a presence of HRS cells in our analysis using a 4-colour assay flow cytometry in the aspirate samples.

According to the WHO classification (2), Hodgkin and Reed-Sternberg cells are almost always positive for CD30 and positive for CD15 in 75-85% of cases. Both CD30 and CD15 are typically present in a membrane pattern increasing in the Golgi area. For B-cell markers, HRS cells are positive for CD20 on a minority of the neoplastic cells with varied intensity in 30-40% of cases, CD79a is less often expressed. The B-cell nature of HRS cells can be demonstrable in approximately 95% of cases by their expression of the B-cell specific activator protein PAX5 and it is usually weaker than that of reactive B cells. Even though HRS cells are usually negative for CD45 (2), the total absence of CD45 is unlikely (13).

CD20 is important in the regulation of human B-cell growth and differentiation. It is present on most mature normal and neoplastic B lymphocytes (14). The prognostic significance of CD20 expression in CHL is controversial and a matter of ongoing debate. A review of the literature showed that the expression of CD20 was not associated with different clinical and laboratory features among equivalently treated patients (14) and has no prognostic significance for the failure-free survival and overall survival in CHL patients (14,15). One study on 248 CHL patients found that failure-free and overall survival were reduced considerably in CD20-positive patients as compared with CD20-negative patients (16). Whereas in another study of 119 CHL patients showed a significantly higher frequency of disease relapses in the CD20-negative group and a better failure-free and overall survival in the CD20-positive group (17).

Previously, bone marrow biopsy (BMB) was the recommended approach for staging in newly diagnosed patients with HL. Recently, positron emission tomography/computed tomography (PET/CT) is required for staging and response assessment in lymphoma according to the recommendations whenever it is available (18-20). Studies have shown that conventional staging can detect bone marrow involvement in only 5–8% of patients, whereas PET/CT staging can detect bone marrow involvement in up to 18% of the cases (20).

Moreover, HL is [18F]fluorodeoxyglucose - avid almost all, thus PET-CT using FDG is more accurate than CT for staging in HL and NHL with increased sensitivity, particularly for extranodal disease (19).

Furthermore, PET/CT leads to change in stage in 10% to 30% of patients, more often upstaging, although alteration in management occurs in fewer patients, with no demonstrated impact on overall outcome (18). El-Galaly *et al.* (12), in a cohort study, investigated whether BMB can add useful information to FDG-PET/CT staging in patients with HL. They concluded that PET/CT can accurately detect marrow involvement therefore the evaluation by BMB is often unnecessary. Likewise, Adams *et al.* made the same observation for the appropriate use of FDG-PET/CT to replace BMB in newly diagnosed HL (22). They also mentioned that the major advantages of FDG-PET/CT over BMB are the fact that it is non invasive and able to visualize the entire bone marrow therefore eliminating any sampling errors. Nevertheless, biopsy is recommended to confirm residual disease and to exclude false-positive uptake with FDG before starting second-line therapy (20).

We reported a case of refractory classical Hodgkin lymphoma in which Hodgkin Reed-Sternberg (HRS) cells were only demonstrated in bone marrow aspirations and trephine biopsy sections only when the disease disseminated. The patient's bone marrow aspirations revealed the typical morphologic characteristics of Reed-Sternberg cells. Whereas, the trephine biopsy sections showed infiltration by scattered Reed-Sternberg cells and mononuclear Hodgkin cells in reactive background. Immunophenotyping analysis by flow cytometry and immunohistochemistry confirmed the presence of HRS cells.

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