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Bone Marrow Biopsy Imprint Preparations: Use for Molecular Diagnostics in Leukemias*

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ABSTRACT

Bone marrow (BM) biopsies occasionally fail to yield aspirate specimens; such "dry taps" pose diagnostic difficulties. In the absence of a BM aspirate, morphological evaluation and cytochemistry rely on core biopsy imprint preparations (IP) and other analyses, e.g., molecular pathology studies, become impossible. The use of imprint preparations (IPs) for molecular diagnostics based on messenger ribonucleic acid (mRNA) analysis by reverse transcription-polymerase chain reaction (RT-PCR) for detection of breakpoint cluster region (bcr) gene rearrangements in chronic myelogenous leukemia (CML) and myeloperoxidase (MPO) mRNA in acute leukemias (AL) is described. Fifteen IPs from five core biopsies (three CML and two AL) were used. Analysis of bcr was positive in all nine IPs from the three cases of CML and was confirmed on peripheral blood samples. Detection of MPO mRNA established the myeloid lineage of the blasts in two cases of AL, when cytochemistry and flow cytometry failed to reveal myeloid markers. These tests are useful when fresh BM aspirate is unobtainable, since multiple imprints of the biopsy are easily prepared at the bedside.

Introduction

Bone marrow (BM) biopsy procedures performed for diagnosis or monitoring of patients with acute or chronic leukemias may fail to yield aspirate specimens. Such cases of "dry tap" are not uncommon at the time of presentation (hypercellular, "packed" marrow) or later during the course of the disease (bone marrow fibrosis, chemotherapy-related bone marrow hypoplasia, etc.). In the absence of aspirate specimens, BM morphology evaluation and cytochemistry must rely on core biopsy sections and imprint preparations (IP) only. Immunophenotyping by flow cytometry, chromosome analysis, gene rearrangement studies, as well as other molecular diagnostic tests are prevented by the lack of BM aspirate. The possibility of utilizing BM imprint preparations for molecular diagnostics has been explored. Detection

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of gene rearrangements at the mRNA level has been demonstrated on BM aspirate or peripheral blood (PB) smears.[8] The method is modified in this study to use biopsy IPs and adapted for mRNA analysis by RT-PCR for detection of (i) bcr gene rearrangements in CML, and (ii) MPO mRNA in AL, for lineage determination of primitive leukemic blasts.

Performing molecular diagnostic tests on cellular material from biopsy IPs is clinically attractive in many situations and offers the only diagnostic modality in cases of dry tap, during patient monitoring after chemotherapy; at this time, leukemic cells may no longer be detected in the PB and residual leukemia in the BM must be ruled out. In such cases, the high sensitivity of the RT-PCR procedure makes possible the detection of minimal residual disease, or confirmation of complete remission at the molecular level, even when BM aspirate specimens are not obtained, and the only material available is the BM core biopsy.

Materials and Methods

Case Identification

The criteria for inclusion in this prospective study were: BM biopsies performed for diagnosis or monitoring of CML or AL and failing to yield BM aspirate specimens. Five cases studied during 1992 and reported by the William Beaumont Hospital Hematology Laboratory met these criteria: three cases of CML and two cases of AL. According to our current BM protocol, multiple IPs were prepared at the bedside, in every case of dry tap. Wright-Giemsa stained IPs were used for morphology evaluation. The extra IPs were air-dried and stored unstained at room temperature until used for cytochemistry, when appropriate. For this study, three of these extra IPs were used in each case, for molecular diagnostics tests.

Morphology Review

All bone marrow biopsies were evaluated for cellularity and myeloid: erythroid (M:E) ratio. Granulopoiesis, erythropoiesis, and megakaryopoiesis were assessed both quantitatively and qualitatively for maturation and dysplasia, increase in immature forms, and percentage of blasts. Reticulin and collagen fibrosis were evaluated by silver impregnation and trichrome stains, respectively. The PB smears were also reviewed for all patients; the 200-cell manual leukocyte differential counts were verified and CBC data reviewed. Dysplastic changes in the BM and PB were assessed according to the criteria of Juneja et al[10] and graded using the modifications of Pugh et al[15] and Crisan et al.[3] Cytochemical stains for MPO, Sudan Black B (SBB), chloroacetate "specific" esterase (CAE), Periodic Acid Schiff (PAS), and "nonspecific" propionate esterase (NSE) were performed on buffy coat preparations of PB and on BM IPs for cases of AL. Leukocyte alkaline phosphatase (LAP) stain and scoring was performed on PB smears in cases of CML or if CML was part of the differential diagnosis. Flow cytometry reports were also reviewed.

Messenger Ribonucleic Acid Analysis

Total cellular ribonucleic acid (RNA) was extracted from material scraped from air-dried, unstained BM IPs, stored at room temperature for one to three days. Three IPs were used in each case, and RNA extraction was performed separately from each IP, using the single-step RNA isolation procedure of Chomczynski and Sacchi[2] as adapted in the commercial RNAzol™ Kit.* Special precautions to avoid slide to slide contamination

* Cinna/Biotecx, Friendswood, TX.

were followed,[8] and each case was studied independently at the time of BM evaluation.

Complementary Deoxyribonucleic Acid Synthesis: Reverse transcription of mRNA to produce first-strand cDNA was performed according to Hanson et al[8] for analysis of bcr and according to Crisan et al[6] foranalysis of MPO mRNA.

Polymerase Chain Reaction (PCR): Target sequences contained in the bcr/abl chimeric cDNA and in MPO cDNA synthesized in the previous step were amplified using PCR with primers specific for bcr and abl[8] or MPO mRNA sequences.[6] The oligonucleotides used as PCR primers were synthesized on a 391 PCR Mate Nucleic Acid Synthesizer† according to the manufacturer's specifications. Positive and negative cellular controls for analysis of bcr were represented by the human leukemia cell lines K562 (Philadelphia chromosome-positive) and HL-60 (Philadelphia chromosome-negative), respectively. The HL-60 cell line, derived from a patient with acute myelogenous leukemia (AML-M2), was also used as a positive cellular control for MPO mRNA expression. Negative cellular controls were represented by normal PB lymphocytes that do not express MPO mRNA. The PCR negative controls (no DNA) were included with each patient sample to rule out contamination with either cDNA or genomic DNA. The commercially available phage DNA template with the appropriate primers‡ was used as positive PCR control. The total cellular RNA extraction and reverse transcription steps were monitored by using internal controls for each patient BM IP: PCR amplification of the normal abl and β actin cDNA sequence in bcr and MPO mRNA analysis, respectively. Validation of PCR amplification products was achieved by size determination and by reamplification using a second pair of nested PCR primers.[6,8] Strict guidelines to avoid false positive PCR results were followed,[11] and all patient sample manipulations and pre-PCR steps were performed in a laboratory physically separated from the PCR work area.

Confirmation of IP Analysis

Both tests for mRNA detection (bcr and MPO mRNA) were also performed on PB samples collected at the time of BM evaluation for confirmation of the results obtained by IP analysis. Additionally, bcr gene rearrangements in CML cases were confirmed by conventional Southern hybridization. Deoxyribonucleic acid (DNA) was extracted from PB leukocytes obtained at the time of BM evaluation. Automated DNA extraction was performed in a Nucleic Acid Extractor,† according to the manufacturer's specifications. The DNA was digested separately with two restriction endonucleases, Bgl II and Xba I,§ and hybridized with a ^{32}P-labeled universal bcr probe‖ following the manufacturer's instructions. The DNAs extracted from the K562 and HL-60 cell lines were used as positive and negative controls, respectively, for analysis of bcr by Southern hybridization.

One case was further investigated at the molecular level, using PB leukemic cells for B and T cell gene rearrangement studies, with ^{32}P-labeled J_H, J_K, and $C_T\beta$ specific probes,¶ and DNA digested separately with three restriction endonucleases, Eco RI, Bam HI, and Hind III§ in a standard Southern blot format. The DNAs extracted from positive patient

† Applied Biosystems, Foster City, CA.
‡ Perkin-Elmer, Norwalk, CT.
§ Bethesda Research Laboratories, Gaithersburg, MD.
‖ TransProbe-1, Oncogene Science, Inc., Uniondale, NY.
¶ Oncor, Inc., Gaithersburg, MD.

TABLE I

Chronic Myelogenous Leukemia Cases: Clinical Data and Peripheral Blood Findings

Clinical Data	*Case 1*	*Case 2*	*Case 3*
Age/Sex	75/M	55/F	83/M
History	Splenomegaly, massive Leukocytosis, marked	4 year history of CML; Under chemotherapy (Hydrea); Moderate anemia	Leukocytosis, marked; Anemia; Thrombocytopenia
Selected hematology data			
Hemoglobin (g/dl)	13.8	8.1	7.7
WBC (bill/L)	165.0	48.4	27.9
Neutrophils (bill/L)	84.13	14.52	23.71
Percent blasts	<1	47.5	0
Monocytes (bill/L)	33.83	1.21	0.28
Basophils (bill/L)	8.25	5.57	0
Platelets (bill/L)	222	703	27
LAP score	226	2	88
Dysplasia (granulocytes)	No	Mild	Mild
Cytochemistry of blasts	ND	MPO, CAE, PAS: negative SBB, NSE: weak to moderate positivity in most blasts	ND
Immunophenotype (Flow cytometry)	ND	80% of mononuclear cells positive for CD 34, CD 13, CD 33, and HLA–DR	ND
Cytogenetics	Ph^1	Ph^1; trisomy 21	Normal
Bcr analysis			
Southern	Positive (Bgl II R: 12.5 kb) (Xba I RR: 14.5; 24 kb)	Positive (Bgl II RR: 8; 4.4 kb) (Xba I R: 12 kb)	Negative (Bgl II, Xba I:G)
RT – PCR	Positive	Positive	Positive

R = gene rearrangement band.
RR = two rearrangement bands found.
G = germline configuration bands only.
kb = kilobase.
Ph^1 = Philadelphia chromosome.
ND = not done.
LAP = leukocyte alkaline phosphatase.
Bcr = breakpoint cluster region.
MPO = myeloperoxidase.
CAE = chloroacetate "specific" esterase.
PAS = periodic acid Schiff.
SBB = Sudan black B.
NSE = "nonspecific" propionate esterase.
RT–PCR = reverse transcription–polymerase chain reaction.

samples, previously analyzed, served as positive controls, and placenta DNA was used as negative control.

Interpretation of Southern analysis for bcr and B/T cell gene rearrangements was based on examination of autoradio-

TABLE II

Chronic Myelogenous Leukemia Cases: Bone Marrow Findings

Bone Marrow Morphology	*Case 1*	*Case 2*	*Case 3*
Cellularity	100%	100%	60%
M:E ratio	> 10:1	> 10:1	> 10:1
Granulopoieses	Increased, mkd	Increased, mkd	Increased, slight
Erythropoiesis	Normal	Decreased	Decreased
Megakaryopoiesis	Increased, clustering	Increased, mkd, with clustering	Normal
Immaturity	No	Increased, mkd	No
Dysplasia	No	Mild–moderate	Mild–moderate
Fibrosis	Increased reticulin, focal, mild	Increased reticulin, mild–moderate	No
Morphology diagnosis	MPD, consistent with CML	CML in blast crisis (myeloid)	Granulocytic hyperplasia with dysplasia; Erythroid hypoplasia
Bcr analysis of IPs (RT–PCR)	Positive	Positive	Positive
Final diagnosis	CML	CML in blast crisis (myeloid)	CML

Bcr = breakpoint cluster region.
IP = imprint preparations.
RT–PCR = reverse transcription–polymerase chain reaction.
M:E ratio = myeloid : erythroid ratio.
MPD = myeloproliferative disease.
CML = chronic myelogenous leukemia.
Mkd = marked.

grams or by imaging. Autoradiograms were developed by exposing the blots to x-ray film at −70°C for two to three days. For imaging, the blots were air-dried and exposed to phosphor screens** for three to 10 hours; the phosphor screens that absorbed the energy emitted by the beta particles in the DNA hybrids were subsequently scanned on a phosphor imaging device,** as per the manufacturer's specifications. The data were digitized, and the image displayed on the computer monitor was examined and printed.

Cytogenetics Studies

Chromosome analysis was performed in all cases on PB specimens only, using the trypsin-Giemsa banding technique. At least 10 metaphases were analyzed for each case.

Results

The findings for PB and BM in the three cases of CML are presented in tables I and II. Cases 1 and 2 were morphologically typical of CML presenting in chronic phase and blast crisis, respectively, and also Philadelphia (Ph^1) chromosome positive. Both cases were confirmed at the molecular level by positivity of the bcr gene rearrangement detected in PB specimens by standard Southern hybridization with a bcr probe specific for the bcr/abl fusion gene that is diagnostic of CML. In Case 2, the lineage of the blasts was established by PB

** Molecular Dynamics, Sunnyvale, CA.

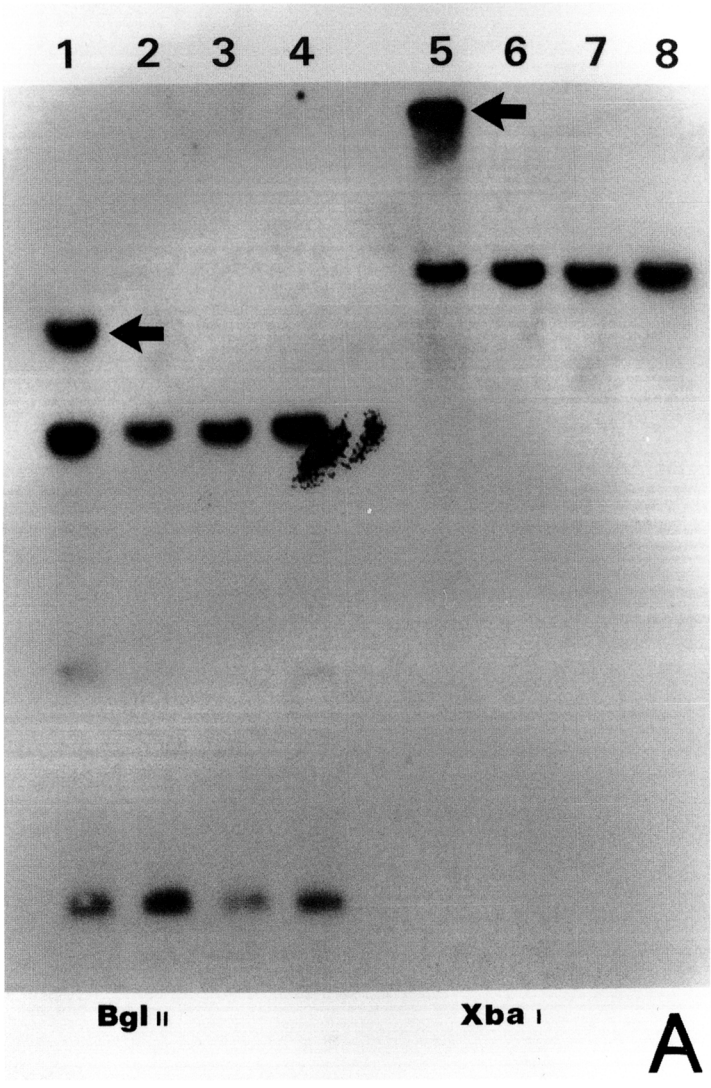

FIGURE 1A. Southern analysis for detection of breakpoint cluster region (bcr) gene rearrangement. The deoxyribonucleic acids (DNAs) in lanes 1 to 4 were digested with Bgl II; the same DNAs were digested with Xba I and loaded in lanes 5 to 8. The blot was hybridized with a ^{32}P labeled bcr universal probe (Oncogene Science, Uniondale, NY). Lanes 1 and 5: positive control; lanes 2 and 6: negative control (Oncogene Science, Uniondale, NY); lanes 3 and 7: CML Case 3; lanes 4 and 8: negative patient sample not pertaining to this study. Unmarked bands represent germline bands common to every DNA: 4.8, 2.3 (faint in photograph but visible on original autoradiogram), and 1.1 kb for Bgl II, and 10 kb for Xba I. Rearrangement bands are indicated by arrows (positive control lanes).

immunophenotyping, allowing the diagnosis of myeloid blast crisis, although the cytochemistry was not contributory. The cause of dry tap in these two cases was hypercellularity at 100 percent; analysis of bcr by RT-PCR was possible on the core biopsy IPs, and both cases were confirmed by bcr gene rearrangement positivity for the BM specimens (table II) as well as PB (table I). Case 3 illustrates the diagnostic usefulness of high sensitivity bcr gene rearrangement tests. The BM and PB findings are not typical of CML, cytogenetics studies failed to reveal a Ph^1 chromosome and bcr analysis of PB granulocytes by standard Southern hybridization was negative (figure 1A). The morphology diagnosis was granulocytic hyperplasia with dysgranulopoiesis and neutrophilia. However, analysis of bcr by RT-PCR detected the chimeric bcr/abl mRNA in PB leukocytes and also in BM IPs (figure 1B), establishing the diagnosis of CML.

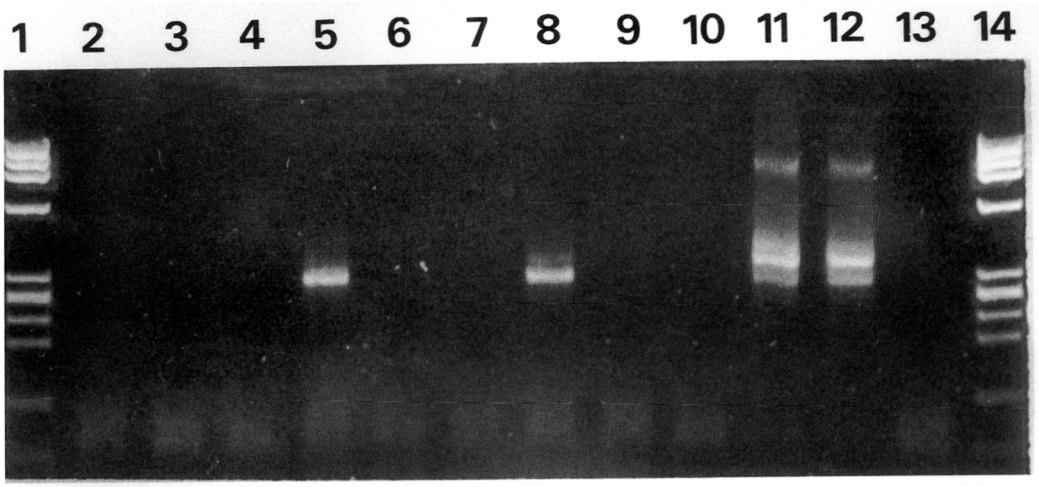

FIGURE 1B. Ethidium bromide stained agarose gel electrophoresis of ΦX174-Hae III size markers (lanes 1, 14), and 1/10th of final products from separate nested polymerase chain reaction (PCR) amplification reaction of cDNA derived from negative cellular controls (lanes 2, 3), negative (no DNA) control (lanes 4, 7, 10, 13), positive cellular control (lanes 11, 12), and bone marrow imprint preparation (IP) and peripheral blood from Case 3 (lanes 5 and 8, respectively), and from bone marrow aspirate and peripheral blood (lanes 6 and 9, respectively) from the same patient shown in panel A, as not pertaining to this study. Positivity is indicated by amplification bands of 225 to 300 bp sizes (lanes 5, 8, 11, 12).

Findings of PB and BM in the two cases of AL are summarized in tables III and IV. Case 1 is unusual in view of the history of essential thrombocythemia, evolving with pancytopenia, circulating blasts and BM focal immaturity. Based on cytochemistry and immunophenotyping of PB blasts, this picture was interpreted as transformation to acute undifferentiated leukemia (AUL). Owing to extensive BM fibrosis, no BM aspirate was obtained, and the only material available for molecular diagnostic studies were core biopsy IPs. The MPO mRNA analysis of IPs, as well as PB blasts, demonstrated positivity and established the myeloid lineage of these primitive blasts and, hence, the final diagnosis of AML-MO evolving from a previously diagnosed essential thrombocythemia. Such transformations are rare,[17] and the possibility of this case representing a CML in blast crisis cannot be ruled out by the negative cytogenetics study; bcr analysis was not requested, but would have been helpful in this situation.

Case 2 presented as an adult type of B cell acute lymphoblastic leukemia (ALL), with virtual replacement of the normal hematopoietic precursors by the leukemic blasts with typical ALL morphology (figures 2A, 2B, and 2C). Both the cytochemistry pattern of positivity and the immunophenotype of the circulating blasts support this diagnosis. This case was extensively studied at the molecular level (table V) owing to the apparent discrepancy between morphology, cytochemistry, and flow cytometry findings which are typical of ALL and the positivity for MPO mRNA, although the cytochemistry of the blasts was MPO-negative (figure 2D). The B and T cell gene rearrangement test confirmed the

TABLE III

Acute Leukemia Cases: Clinical Data and Peripheral Blood Findings

Clinical Data	*Case 1*	*Case 2*
Age/Sex	60/F	46/M
History	3 year history of essential thrombocythemia; presenting with pancytopenia	Weight loss, splenomegaly, anemia, leukocytosis with circulating blasts
Selected hematology data		
Hemoglobin (g/dl)	7.6	12.3
WBC (bill/L)	1.9	20.0
Neutrophils (bill/L)	0.61	0.90
Percent blasts	14	65.5
Monocytes (bill/L)	0.08	0.10
Lymphocytes (bill/L)	0.92	5.80
Platelets (bill/L)	13	24
Cytochemistry of blasts	MPO, CAE, PAS: negative SBB, NSE: weakly positive in rare blasts	MPO, SBB, CAE: negative PAS, NSE: positive
Immunophenotype of blasts	CD 34 positive, all other markers, negative; early blasts expressing no specific lineage	CD 34, CD 19, CD 20, CD 10 (CALLA): positive consistent with ALL
Cytogenetics	Normal	Ph^1
MPO mRNA	Positive	Positive

Ph^1 = Philadelphia chromosome.
MPO = myeloperoxidase.
PAS = periodic acid Schiff.
NSE = "nonspecific" propionate esterase.
mRNA = messenger ribonucleic acid.
CALLA = common ALL antigen.
CAE = chloroacetate "specific" esterase.
SBB = Sudan black B.
ALL = acute lymphoblastic leukemia.

presence of a monoclonal population of B cells (table V). A bcr gene rearrangement study by mRNA analysis (RT-PCR) was positive on PB and IP blasts and confirmed on PB by Southern hybridization, and later by the cytogenetic finding of a Ph^1 chromosome. Both molecular diagnostic tests (RT-PCR, Southern) demonstrated the type of bcr gene rearrangement diagnostic of CML. This allowed reclassification of this patient's diagnosis as CML presenting in blast crisis of lymphoid lineage, as opposed to *de novo* ALL. Interestingly, a follow-up BM study after chemotherapy with an ALL regimen confirmed complete remission at the morphological and cytogenetic levels. No bcr positivity was found by RT-PCR or Southern analysis, and B cell gene rearrangements were no longer found, confirming remission at the molecular level as well (table V).

Discussion

Imprints of BM core biopsies can be successfully used for molecular diagnostics testing, based on mRNA analysis. The cellular material scraped from glass

TABLE IV

Acute Leukemia Cases: Bone Marrow Findings

Bone Marrow Morphology	*Case 1*	*Case 2*
Cellularity	> 90 %	100 %
Granulopoiesis	Decreased	Virtually absent
Erythropoiesis	Decreased	Virtually absent
Megakaryopoiesis	Decreased with clustering	Virtually absent
Immaturity	Increased, focal	100 %
Fibrosis	Increased reticulin, mkd.	No
Morphology diagnosis	Essential thrombocythemia evolving with extensive reticulin fibrosis and focal immaturity; Severe pancytopenia with circulating blasts; Consistent with transformation to AUL	ALL (B-cell)
MPO–mRNA (IPs)	Positive	Positive
Final diagnosis	AML–MO	CML in blas crisis (lymphoid)

ALL = acute lymphoblastic leukemia. CML = chronic myelogenous leukemia.
MPO–mRNA = myeloperoxidase–messenger ribonucleic acid.
IP = imprint preparations. AUL = acute undifferentiated leukemia.
Mkd = marked.

slides of air-dried, unstained BM biopsy imprints is used for RNA extraction and reverse transcription. First-strand cDNAs are generated, corresponding to multiple mRNA species. From the cDNA stage on, the procedure can be adapted for detection of specific mRNAs by PCR amplification of cDNA with primers designed to flank the cDNA (mRNA) sequences of interest. This RT-PCR procedure has been previously used for mRNA analysis of fresh or cryopreserved specimens and also hematology smears.[6,8] The procedure described here was adapted for use of bone marrow IPs in the detection of bcr gene rearrangements and MPO mRNA expression.

Analysis of bcr is now routinely used in clinical hematology to confirm the diagnosis of CML, to establish the diagnosis in Ph^1-negative cases, and to rule out CML in cases of myeloproliferative disorders or chronic myelomonocytic leukemia, that may mimic CML.[4,18] An important utilization of this molecular diagnostic test is in monitoring CML patients who undergo chemotherapy.[5,13,18] The use of PCR-based methods has increased the detection sensitivity considerably and permitted better evaluation of residual leukemia and diagnosis of minimal residual disease or confirmation of complete remission, early detection of relapse, as well as monitoring BM transplant patients and assessing the efficiency of autologous BM purging of bcr-positive cells.[7,9,12,16] The diagnostic use of analysis of bcr on BM IPs is illustrated by the three cases of CML studied: diagnosis confirmation (Case 1 and 2), and establishing the diagnosis in Case 3, with atypical morphology and cytogenetics

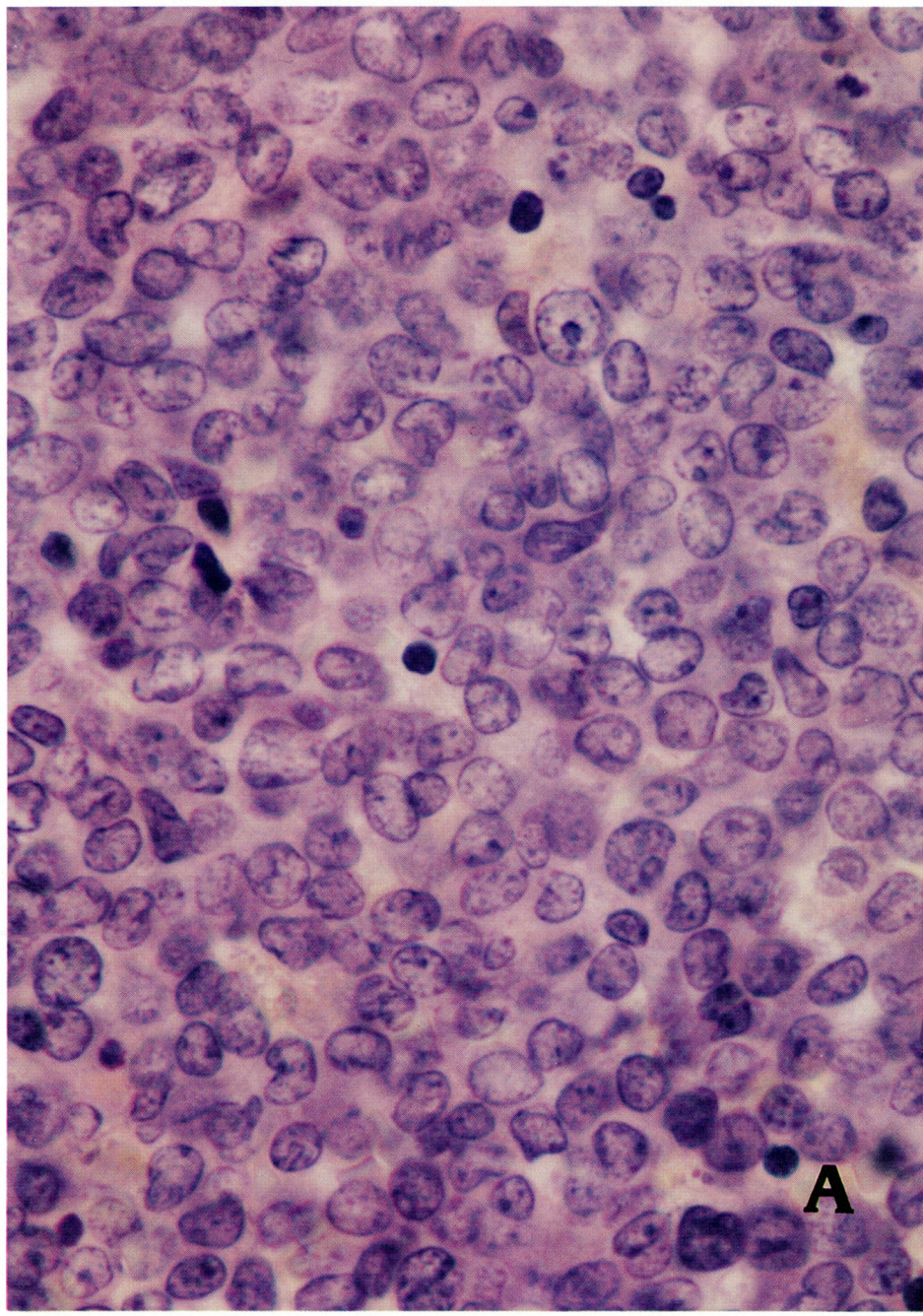

FIGURE 2A. Bone marrow core biopsy (hematoxylin & eosin, 500 × magnification) of patient presenting as acute lymphocytic leukemia (ALL); normal bone marrow is virtually replaced by blasts.

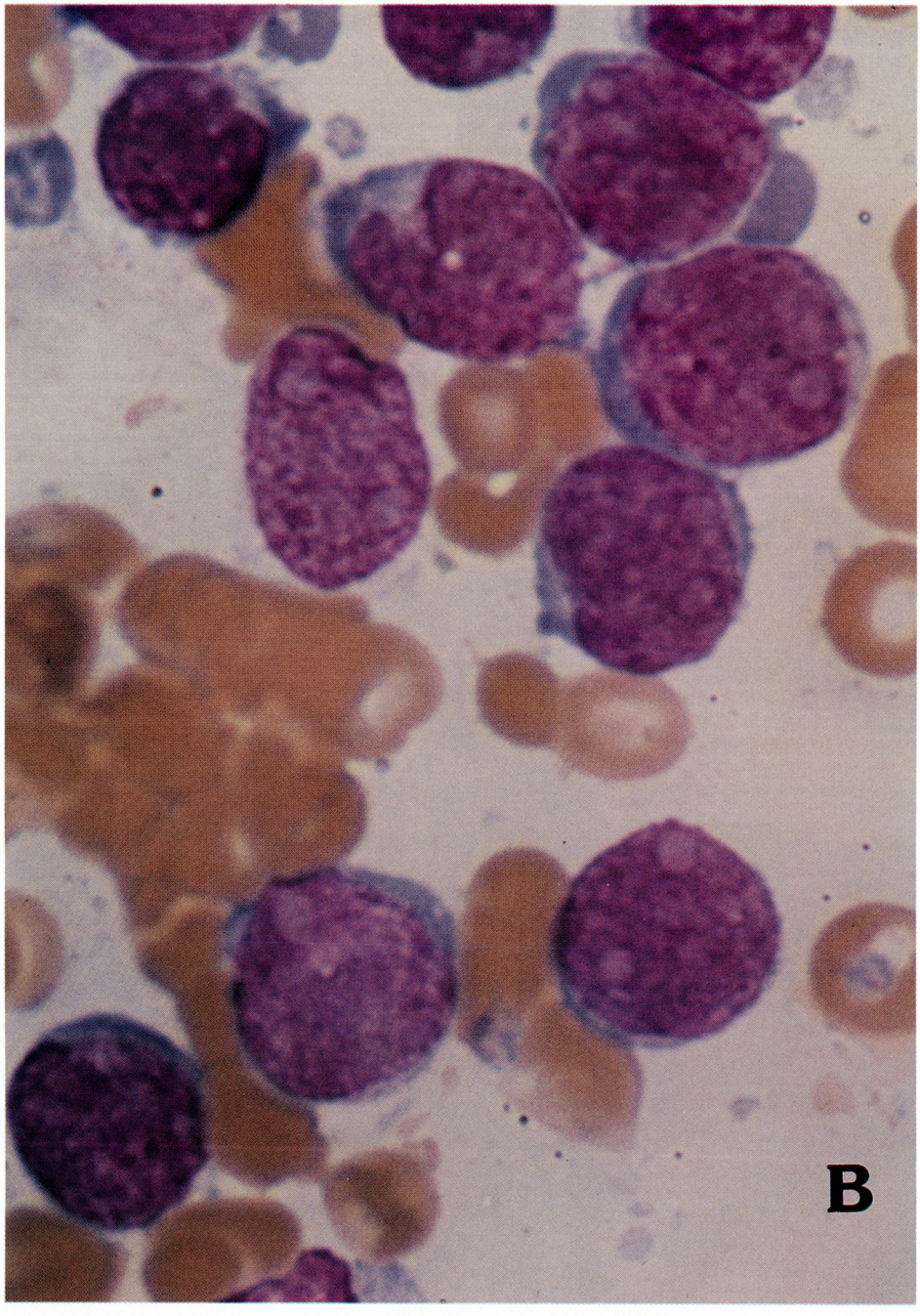

FIGURE 2B. Wright-Giemsa stain of bone marrow core biopsy imprint, composed entirely of blasts with high N/C ratio, fine chromatin with one or more prominent nucleoli, and scant agranular cytoplasm.

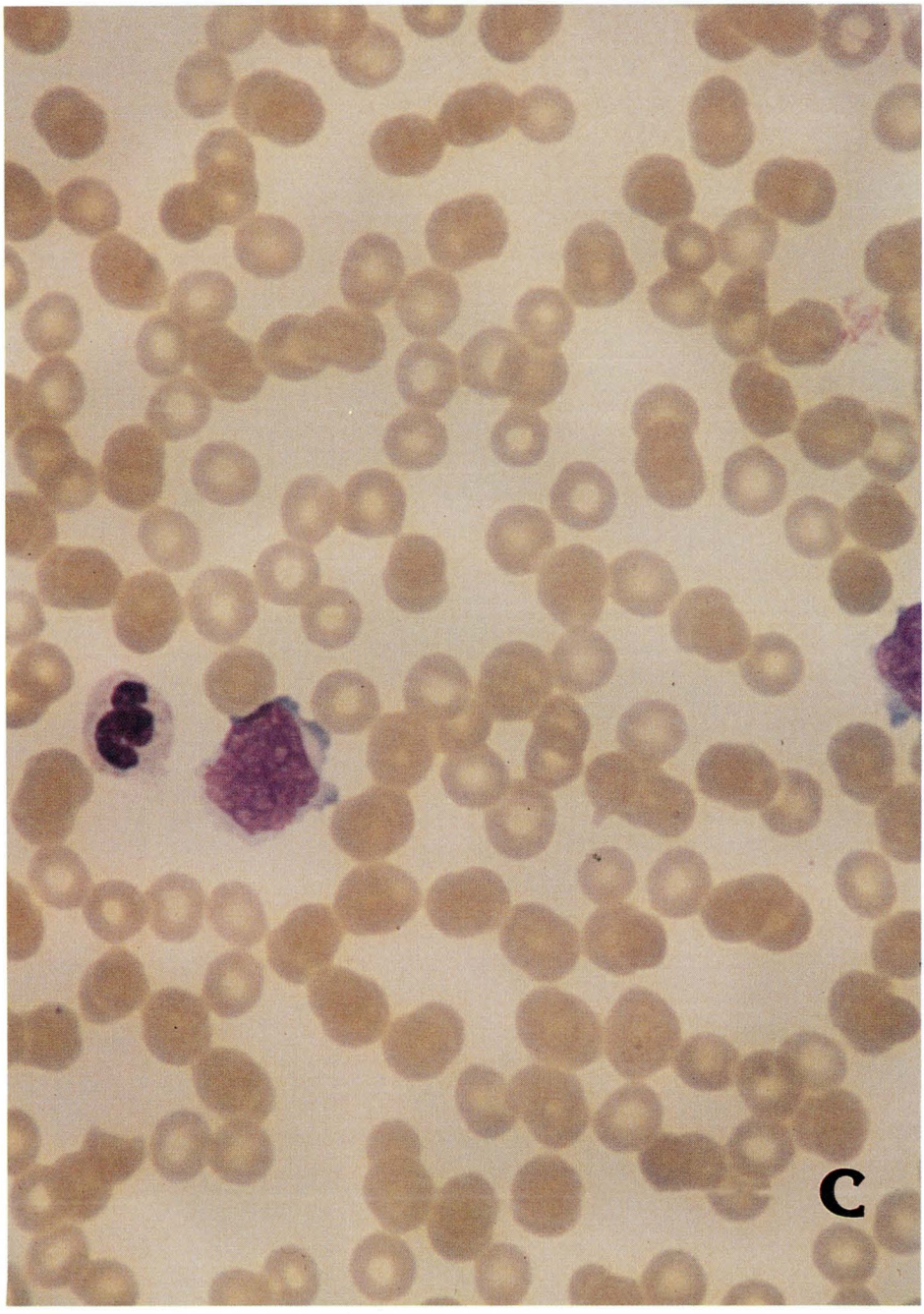

FIGURE 2C. Peripheral blood smear, Wright-Giemsa stain, demonstrates the presence of blasts similar to those found in the marrow.

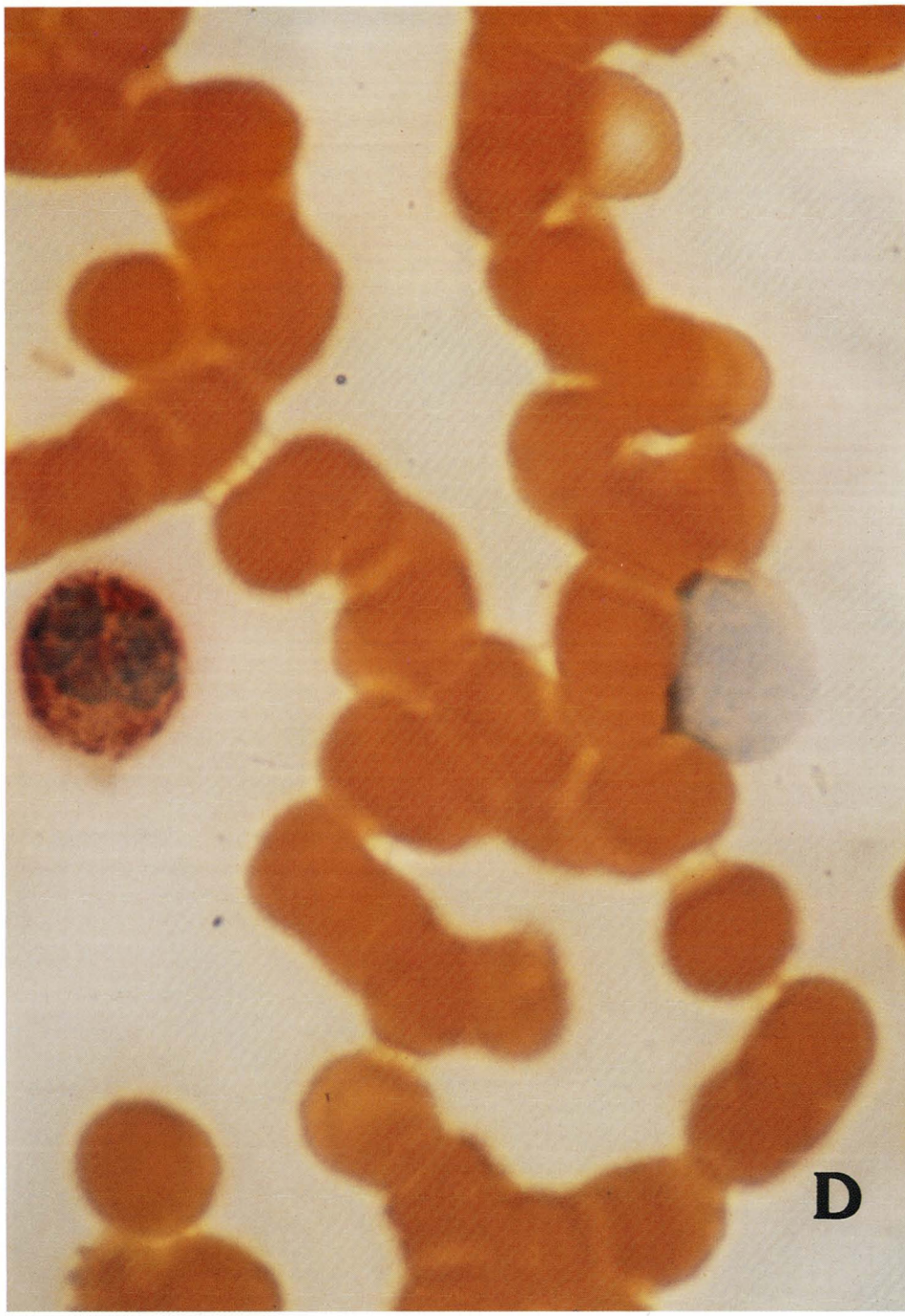

FIGURE 2D. Myeloperoxidase stain of bone marrow imprint preparation of the core biopsy demonstrates a negative blast and a mature granulocyte as a positive internal control.

TABLE V

Molecular Diagnostic Tests on Peripheral Blood and Bone Marrow of a Patient Presenting as Adult Type Acute Lymphoblastic Leukemia (Case 2), Diagnosed as Chronic Myelogenous Leukemia in Lymphoid Blast Crisis

Test	*Presentation*	*Post–chemotherapy (Complete Remission)*
MPO mRNA (RT–PCR) on circulating blasts	Positive	ND (no blasts)
MPO mRNA (RT–PCR) on BM IPs	Positive	ND (no blasts)
Bcr analysis		
– Southern (bcr specific probe) on PB	Positive (Bgl II RR: 6.3, 4.2 kb) Xba I RR: 13.3, 4.1 kb)	Negative (Bgl II and Xba I: G)
– RT–PCR (CML specific)		
– BM IPs	Positive	Negative
– PB	Positive	Negative
B/T cell gene rearrangements (PB)	Monoclonal B cell population (J_H, Eco RI RR: 9.7; 7.9 kb J_H, Bam HI R: 14 kb J_H, Hind III RR: 6.2; 3.6 kb)	Negative (Eco RI, Bam HI, & Hind III: G)

MPO–mRNA = myeloperoxidase–messenger ribonucleic acid.
RT–PCR = reverse transcription–polymerase chain reaction.
Bcr = breakpoint cluster region.
CML = chronic myelogenous leukemia.
BM = bone marrow.
PB = peripheral blood.
IP = imprint preparations.
G = germline confiburation bands only

findings. The higher sensitivity of the RT-PCR procedure made the diagnosis possible even when standard Southern analysis failed to reveal bcr gene rearrangement. This superior sensitivity also makes the procedure valuable for future monitoring of these patients and essential for the follow-up of the Ph^1-negative, Southern-negative Case 3.

The second molecular diagnostic test applied to BM IPs addresses expression of the MPO gene in myeloid precursors. Myeloperoxidase is considered the definitive marker for myeloid lineage. In the French-American-British classification of AL, MPO positivity in $\geq$3 percent of the blasts is diagnostic of AML, regardless of other phenotypic markers.[1] Enzyme levels of MPO parallel early myeloid maturation, while levels of MPO mRNA decrease or even disappear with maturation.[14,19] This makes MPO mRNA a good marker for establishing the myeloid lineage of primitive leukemic blasts that are cytochemically negative for the enzyme and immunophenotypically negative for myeloid markers. This marker may also be used to confirm the myeloid lineage

when the percent of MPO-positive blasts is borderline or the intensity of MPO positivity is weak or questionable and immunophenotyping is noncontributory.

An RT-PCR procedure for detection of MPO mRNA in fresh or cyropreserved cells was previously developed.[6] The procedure is adapted for use of BM imprints in this study. Myeloid lineage is established in both AL cases, although the leukemic blasts are morphologically non-myeloid (primitive or lymphoid), cytochemically negative for MPO and no positivity for myeloid markers is found by flow cytometry on PB samples. The same method applied to leukemic blasts from fresh PB samples confirms positivity for MPO mRNA, serving as procedural control for the BM IP findings. The value of in-depth molecular studies for establishing a correct diagnosis is illustrated by the second AL case (table V), presenting as an adult type of B cell ALL, while representing in fact, CML in lymphoid blast crisis at the time of presentation. This case also illustrates the usefulness of molecular diagnostics tests for patient monitoring after chemotherapy to confirm complete remission at the molecular level.

The diagnostic advantages of the RT-PCR procedure for mRNA analysis are multiple. It can be used at the time of initial diagnosis in CML and AML, and especially for patient monitoring following therapy (chemotherapy, BM transplant). The mRNA analysis of BM imprints is the only diagnostic modality when BM aspirate is unobtainable (dry tap), the leukemic cells are no longer detected in the PB, and the BM biopsy is performed to evaluate for residual leukemia. In such cases, the morphological examination of the core biopsy sections may pose a difficult diagnostic problem in distinguishing between residual leukemic blasts and focal immaturity as seen in recovery marrow after chemotherapy. The presence of bcr-positive cells for example, would diagnose residual leukemia, or their absence would confirm molecular remission, as in the case presented in table V.

The technical advantages of the RT-PCR based methods are also multiple. The high specificity and sensitivity are conferred by PCR amplification of cDNA and make possible the use of small numbers of cells. The analysis in our study was possible even when only one IP was used. The ease, simplicity, and safety (no radioactivity used) are additional advantages. The turnaround time is drastically reduced from the five to 10 days necessary for Southern analysis to six to eight hours. Thus, the results become available at the time of BM sign-out, which is an important improvement in molecular diagnostics. The BM IPs used in this prospective study are usually one to three days old. The possibility that archival IP preparations stored in most hematology laboratories may be used for molecular diagnostics testing merits investigation. Another important advantage is the convenience of use, since multiple BM IPs may be prepared at the bedside when BM aspiration fails. Similarly, when the need for molecular diagnostic testing becomes apparent, it is possible that fresh or cryopreserved specimens are no longer available for conventional DNA or mRNA analysis methods, and BM IPs represent the only cellular material for molecular diagnostics.

Acknowledgment

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