

PHILADELPHIA CHROMOSOME-POSITIVE ACUTE MYELOID LEUKEMIA: CYTOIMMUNOLOGIC AND CYTOGENETIC FEATURES

Antonio Cuneo,* Augustin Ferrant,^o Jean-Louis Michaux,^o Hilde Demuyneck,[#] Marc Boogaerts,[#] Andries Louwagie,[@] Chantal Doyen,[^] Michel Stul,[§] Jean-Jacques Cassiman,[§] Paola Dal Cin,[§] Gianluigi Castoldi,* Herman Van den Berghe[§]

*Institute of Hematology, University of Ferrara, Ferrara, Italy; Departments of Hematology, U.C.L. ^oin Brussels and [^]in Mont-Godinne; [§]Center for Human Genetics and Flanders Institute of Biotechnology, and [#]Department of Hematology, University of Leuven; [@]Department of Hematology, A.Z. S. Jan, Brugge, Belgium

ABSTRACT

Background. Little is known about the morphological and clinical features of the minority of acute myeloid leukemias (AML) that carry the t(9;22)(q34;q11) translocation.

Materials and Methods. Cytologic, cytogenetic and clinical features were studied at diagnosis and during disease evolution in 11 patients presenting with *de novo* AML. Diagnoses according to the FAB criteria were AML-M2 (3 cases), AML-M0 and AML-M4 (2 cases each), AML-M4eos, AML-M5, AML-M6, AML-M7 (1 case each).

Results. Immunophenotyping disclosed positivity for CD33 or CD13 and for the CD34 stem cell antigen in all cases tested. Lymphoid-associated markers (LM) were detected in 7/9 patients. In 5 cases the expression of at least 2 LM was seen. In addition, evidence of clonal rearrangement of the immunoglobulin (Ig) and/or T-cell receptor (TCR) genes was documented in 3/4 evaluable cases. The Ph chromosome was found as the sole change in 5/11 cases; the karyotype reverted to normal in 2/4 patients who achieved complete remission. Rearrangement in the M-bcr region was detected by Southern blotting in 2/7 cases.

Conclusions. This infrequent cytogenetic subset of *de novo* AML appears to be characterized by heterogeneous cytologic features, with frequent expression of lymphoid markers, and by unfavorable prognosis. The combination of clinical, cytologic and cytogenetic studies is important in distinguishing *de novo* AML with the t(9;22) from chronic myelogenous leukemia blast crisis.

Key words: acute myeloid leukemia, chromosomes, immunology

A distinct minority of adult acute myeloid leukemias (AML) carry the t(9;22)(q34;q11) translocation, which is cytogenetically indistinguishable from the classical Ph chromosome.¹

Partly due to its rarity, little is known about the morphologic, immunologic and clinical features of this cytogenetic subset of AML. While some patients were classified as AML,² multiple cell lineage involvement as well as mixed-lineage characteristics were detected in other cases.^{3,4} Lineage switch from acute lymphoblastic leukemia to AML has also been described.⁵

The aim of the present report is twofold: a) to analyze cytoimmunologic features in 11 Ph-positive patients with unequivocal AML on morphologic grounds; b) to study cytogenetic findings at presentation and during disease evolution believed to be important in distinguishing this disorder from other Ph-positive diseases.

Patients and Methods

Eleven cases with the Ph chromosome have been found among approximately 700 patients with a diagnosis of AML referred to the Genetic

Correspondence: Prof. Dr. Herman Van den Berghe, Center for Human Genetics, Herestraat 49, B-3000 Leuven, Belgium. Tel. international +32.16.345877. Fax international +32.16.345992.

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| Case | Sex/Age | Cytology ^a | Immunophenotype ^b | Cytogenetics and molecular genetics |
|------|---------|--|---|-------------------------------------|
| 1 | M/32 | AML-M2 | CD34+, CD13+, CD33+ ⁺ , CD14+/-, TdT+ ⁺ , CD10+ ⁺ , CD24+ ⁺ , CD33/CD10+ ⁺ * | Ph+others, M-bcr G, IgH G, TCRβ R |
| 2 | M/33 | AML-M4 | CD34+ ⁺ , CD33+ ⁺ , CD13+, CD14+, TdT+ ⁺ , CD19+ | Ph, M-bcr G, IgH R, TCRβ R |
| 3 | F/47 | AML-M6; prominent dyserythropoiesis. Hyperplasia of the granulocytic lineage | CD34+ ⁺ , CD33+ ⁺ , CD13+/-, CD15+ ⁺ , CD41+/-, CD2+ ⁺ , CD7+, CD24+/- | Ph+others, M-bcr G, IgH G, TCR G |
| 4 | F/52 | AML-M2 | CD34+, CD33+ ⁺ , CD13+ ⁺ , CD10+ ⁺ , TdT+ | Ph, M-bcr G, IgH R, TCRβ R |
| 5 | F/68 | AML-M7 with minor population of lymphoid-like blasts | CD33+ ⁺ , CD41+, CD7+ ⁺ , TdT+ ⁺ | Ph+others, M-bcr R, IgH NA, TCR NA |
| 6 | F/71 | AML-M4 | CD34+, CD33+ ⁺ , CD13+ ⁺ , CD14+/-, CD41+/-, CD7+/-, CD19+ ⁺ | Ph, M-bcr R, IgH NA, TCR NA |
| 7 | F/63 | AML-M0 | CD34+, CD33+ | Ph+others, M-bcr NA |
| 8 | F/73 | AML-M2 | NA | Ph, M-bcr NA, IgH NA, TCR NA |
| 9 | M/50 | AML-M5 | NA | Ph+others, M-bcr G, IgH NA, TCR NA |
| 10 | M/64 | AML-M4 eos | CD33+ ⁺ | Ph+others, M-bcr NA, IgH NA, TCR NA |
| 11 | M/27 | AML-M0 | CD33+ ⁺ , CD10+ | Ph, M-bcr NA, IgH NA, TCR NA |

Table 1. Cytoimmunologic and cytogenetic features in 11 patients with Ph-positive AML

Legend. *double fluorescence; ^amorphologic and cytochemical analysis according to the FAB criteria; ^bother tested markers negative; BC: blast crisis; NA: not available; G: germline; R: rearranged; +/-: 10-20% positive; +: 20-40% positive; ++: 40-60% positive; +++: >60% positive cells in the blast gate.

Center, Leuven, and the Institute of Hematology, Ferrara, for cytogenetic analysis since 1985. All patients presented with *de novo* AML and had no history suggestive of antecedent CML chronic phase or other myeloproliferative disorder.

Remission induction protocols included an anthracycline drug plus cytarabine in conventional doses. Complete remission was defined by the presence of less than 5% BM blasts with a blood count showing more than $1 \times 10^9/L$ neutrophils and more than $100 \times 10^9/L$ platelets. Persistence of 5-20% BM blast cells was classified as partial response (PR).

Cytoimmunologic, cytogenetic and molecular genetic studies

Retrospective examination of bone marrow (BM) and peripheral blood (PB) smears stained by May Grünwald-Giemsa and by routine cytochemical reactions was carried out at diagnosis

and at relapse. The patients were classified according to the FAB criteria.⁶ Cytofluorimetric study of the blast cell phenotype was performed as previously described.⁷ CD34 stem cell marker testing was begun in 1988 (6 patients).

Cytogenetic analysis was conducted at diagnosis, in complete remission and at relapse using a synchronization technique with methotrexate and bromodeoxyuridine or thymidine. The presence of a rearrangement involving the major breakpoint cluster region on chromosome 22 was assessed in 7/11 cases. Not all patients were investigated molecularly because in the 1980s these tests were not performed routinely in every patient. The configuration of the genes coding for the immunoglobulin heavy chain (IgH) and for the β and γ subunits of the T-cell receptor (TCR) was studied in 4 cases in which representative BM samples were available. Molecular investigation methods at our Institutions have been detailed elsewhere.⁷

Results

Cytoimmunologic findings and configuration of IgH and TCR genes

Cytoimmunologic findings are summarized in Table 1. Different FAB classes were represented in this series as follows: AML-M2: 3 cases; AML-M0 and AML-M4: 2 cases each; AML-M4eos, AML-M5, AML-M6, AML-M7: 1 case each.

Immunophenotypic data were available in 9/11 patients. At least one myeloid marker was found in all cases. The CD34 antigen was positive in all 6 cases tested. Lymphoid-associated markers (LM) were seen in 7 cases, whereas a purely *myeloid phenotype* was detected in patients #7 and #10. In 5 cases (#1 through #5), at least 2 LM were seen.

In 3/4 patients tested the IgH and/or TCR genes were in a rearranged configuration. The κ and λ light chain genes were in a germline configuration.

Analysis of hematologic features in 6 patients in CR or PR phase did not disclose hyperplasia of the granulocytic lineage or leukocytosis.

The leukemia FAB type did not change at relapse in 4 patients who achieved CR.

Clinical features

Salient hematologic parameters are shown in Table 2. Patient age ranged between 27 and 73 years, median 52.

Median WBC count at presentation was $117 \times 10^9/L$, range 25-324. Mild to moderate splenomegaly was observed 3 cases (#5, #6, #9), whereas massive splenic enlargement was recorded in patient #3.

CR was achieved in 4/11 cases, with a duration range of 3-14 months. Median survival was 7 months. One patient (#1), transplanted in first CR, relapsed 4 months after autologous stem cells were reinfused.

Cytogenetic findings and M-BCR status

Results are detailed in Table 3. In 10/11 cases only abnormal metaphases were detected. Both chromosomally abnormal and normal cells were seen in the remaining patient (#11). The 9;22 translocation was found to be the sole aberration at diagnosis in 5/11 cases. Additional abnormalities, present in 6 patients, involved

Table 2. Hematologic features in 11 patients with Ph-positive AML

| Case | WBC $\times 10^9/L$ Plts $\times 10^9/L$ Hb (g/dL) | %BMbls/ %PBbls | Spleno- megaly | Therapy, outcome* | Survival (months) |
|------|--|-------------------|-------------------|-----------------------------------|----------------------|
| 1 | 77/74/12.6 | 41/51 | no | DNR+Ara-C; Mx+Ara-C, CR, ABMT (7) | 14 |
| 2 | 221/64/11.1 | 65/74 | no | DNR-Ara-C, Hd-AraC NR | 3 |
| 3 | 171/28/11.5 | 35** /41 | yes | DNR+Ara-C NE | ED |
| 4 | 117/68 /10.5 | 68/62 | no | DNR+Ara-C, HDARA-C NR, VPD+CTX NR | 11 |
| 5 | 29/98/10.7 | 58/51 | yes | DNR+Ara-C PR | 8 |
| 6 | 108/ 49/9.3 | 76/81 | yes | DNR+Ara-C, CR (5) | 7 |
| 7 | 76/98/ 12 | 75/71 | no | DNR+Ara-C, CR (3) | 5 |
| 8 | 25/120/11.5 | 81/65 | no | DNR+Ara-C, CR (14) | 17 |
| 9 | 324/2000/7 | 99/98 | yes | DNR+Ara-C NR | 1 |
| 10 | 200/50/8.5 | 76/78 | no | DNR+Ara-C, PR | 7 |
| 11 | 129/54/9.3 | 84/79 | no | VCR+ DNR+ Ara-C NR | 32 |

* duration of CR in parentheses; ** blasts calculated over non erythroid cells; ABMT: autologous BM transplantation; DNR: anthracycline drug; Mx: Mitoxantrone; Ara-C: Cytarabine; HDARA-C: high-dose cytarabine; VPD: Vincristine, Daunorubicin, Prednisone; CTX: Cyclophosphamide; NE: not evaluable, ED: early death CR: complete remission, NR: non responder, PR: partial responder.

chromosomes 8, 10, 19 (2 cases each of trisomy). Additional copies of the Ph chromosome were seen in 2 cases. Structural changes involving 2q, 7q, 9p, 9q, 11p, 17q were found in 1 patient each. The karyotype reverted to normal in 2/4 patients in CR, whereas 40% and 10% residual Ph-positive metaphases were detected in 2 patients (#6, 8), respectively.

Rearrangement of the M-bcr region was detected in 2/7 patients tested. In the remaining 5 cases the M-bcr region was in germline configuration.

Discussion

We studied cytoimmunologic and chromosome findings in a relatively rare cytogenetic subset of AML, representing 1-2% of approximately 700 *de novo* AMLs seen at our Institutions over a 10-year period. This condition is probably under-reported in the literature and consequently cytologic, cytogenetic and

Table 3. Karyotypes in 11 patients with Ph-positive AML.

| Case | Karyotype (No. of cells) | No. abn/total | Karyotype in CR |
|------|--|---------------|--------------------|
| 1 | 46,XY, t(11;9) (9;22) (p12;p12 q34;q11) | 11/11 | normal 100% |
| 2 | 46,XY,t(9;22)(q34;q11)* | 10/10 | No CR |
| 3 | 51,XX, +6, t(9;22)(q34;q11), +10, +19, +der(22)t(9;22)x2 / 52, XX, idem +8 | 16/16 | No CR |
| 4 | 46,XX, t(9;22)(q34;q11) | 10/10 | No CR |
| 5 | 46,XX, t(9;22)(q34;q11), t(2;9)(q12;q33) | 15/15 | No CR |
| 6 | 46,XX, t(9;22)(q34;q11) | 12/12 | normal 60% |
| 7 | 53,XX, t(1;4)(p?;q?) +8, t(9;22)(q34;q11), +10, +14, +19, -20, +21, +21, +der(22)t(9;22)x2 | 10/10 | normal 100% |
| 8 | 46,XX, t(9;22)(q34;q11) | 11/11 | normal 90% |
| 9 | 46, XY, t(9;22)(q34;q11) / 46,XX, idem, t(7;17)(q31;q11) | 15/15 | No CR |
| 10 | 46,XY, t(9;22)(q34;q11), inv(16)(p13q22) | 10/10 | No CR |
| 11 | 46,XY, t(9;22)(q34;q11) | 3/15 | No CR |

*non-clonal structural and numerical abnormalities in addition to t(9;22); CR: complete remission.

clinical features associated with this chromosome abnormality are only partially known.

Although some patients included in this study were diagnosed in the 1980s when immunologic and molecular genetic studies were not performed routinely in all cases, analysis of our data could contribute to a better definition of the hematologic features of Ph-positive *de novo* AML.

In principle, no single clinical or hematologic feature distinguishes Ph-positive leukemia from CML blast crisis in an individual patient;⁸ however, it was unlikely that our patients suffered from undetected chronic phase CML, not only for the absence of a clinical history suggestive of an antecedent hematologic disorder, but for the following considerations as well. Clinically, most of these patients showed either no palpable spleen or minimal splenomegaly. In addition, a classical hematologic picture of chronic or accelerated phase CML was not observed in the

patients who responded to induction therapy. The type of additional changes did not help in distinguishing *de novo* AML from CML blast crisis, but cytogenetic studies disclosed other abnormalities in addition to t(9;22) in only 6/11 cases and, more importantly, the karyotype reverted to normal in at least 60% of metaphases in all 4 responding patients. These data would be very unusual in CML blast crisis, where additional changes are found in approximately 80% of cases⁹ and a majority of residual Ph-positive cells are usually found after administration of chemotherapy.^{1,9} Thus, we can conclude that all 11 cases had *bona fide* AML.

Morphologic studies in our patients showed that besides leukemia with minimal myeloid differentiation, most FAB subtypes of AML may present with the Ph chromosome.

Interestingly, immunophenotyping disclosed a high incidence of inappropriate expression of LM and only 2/9 evaluable patients presented a *purely myeloid* phenotype. In addition, the CD34 stem cell marker was expressed in all cases tested. These findings are in line with previous reports describing some patients with Ph-positive AML and lineage promiscuity.³⁻⁵ Unfortunately, cytoplasmic detection of differentiation antigens was not performed in this study, nor were anti-myeloperoxidase or anti-lysozyme MoAbs used, thus precluding retrospective application of new scoring systems for the diagnosis of biphenotypic leukemia.¹⁰ It is worth noting, however, that 2 or more LM were detected in 5/9 patients (#1-5) who also frequently displayed clonal rearrangement of IgH or TCR genes. These patients would fulfill the criteria for diagnosis of hybrid acute leukemia according to Gale and Ben Bassat.¹¹ Although cytologic features are heterogeneous in this leukemia, most patients have in common an inappropriate expression of LM and/or display clonal rearrangement of IgH and TCR genes. Prospective studies employing a standardized panel of immunologic reagents¹⁰ will probably be able to demonstrate beyond doubt that a diagnosis of biphenotypic AML would be more appropriate in these patients.

Our patients presented with high leukocyte count and, despite their relatively young age,

infrequently achieved CR with conventional chemotherapy. Early relapses invariably occurred in this series, accounting for the observed short survival.

Circumstantial evidence in this analysis and in previous studies^{2,12} would suggest that these patients are to be considered at *high risk* of treatment failure or early relapse. Ongoing trials will be able to define more exactly the prognostic significance of this rare cytogenetic abnormality in AML.^{13,14}

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