Acute Leukemias

Acute erythroid neoplastic proliferations. A biological study based on 62 patients

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Background and Objectives. The terms acute erythroleukemia and AML-M6 are defined in the FAB classification as proliferations of dysplastic erythroid elements mixed with blasts of myeloid origin, but pure erythroid leukemias are not included. The recent WHO classification has a category of acute myeloid leukemia not otherwise categorized, which includes acute erythroid leukemia (M6) of two subtypes: M6a-erythroleukemia (erythroid/myeloid) and M6b-pure erythroid leukemia. The aims of this co-operative study were to discover the incidences of these different subtypes, and pay special attention to the morphology of these entities.

Design and Methods. We reviewed a series of 62 patients with erythroid neoplastic proliferations. Previous medical history, age, sex, peripheral blood and bone marrow cell counts, cytochemical stains, immunophenotype, and cytogenetics were evaluated at presentation. We analyzed the incidence of erythrocyte, leukocyte and platelet abnormalities in the peripheral blood. In bone marrow we analyzed dysplastic features of erythroblasts, granulocytic elements and the megakaryocytic lineage.

Results. Fifty-three patients met the criteria of M6a subtype of the WHO classification, and 2 were classified as having pure erythremia (M6b); 7 cases could not be classified according to the WHO criteria. Fifty-five patients presented with de novo acute leukemia, and seven patients had secondary acute leukemia. The most frequent dysplastic features in blood smears were: schistocytes, tear-drop and pinched cells in erythrocytes; hypogranulation and gigantism in granulocytic series, and micromegakaryocytes and unconnected nuclei in megakaryocytes were the most dysplastic features. A positive PAS reaction and increase of bone marrow iron with ring sideroblasts were common features. Trilineage dysplasia was present in 54% of cases. Dysplastic features in granulocytic elements were absent in 26% of patients and minimal erythroidlastic dysplasia was observed in seven patients. A complex karyotype was seen in 27% of patients; chromosomes 5 and 7 were the most frequently involved.

Interpretation and Conclusions. De novo acute erythroid leukemia was more frequent than secondary cases in our series. The most frequent type of acute erythroid proliferation was the WHO M6a subtype and the least was the pure erythroid leukemia. We found a group of seven patients (11%) who could not be classified according to the WHO criteria. Morphologic findings of erythrocytes in peripheral blood, such as schistocytes, tear-drop and pinched cells, were outstanding features. Morphologic aspects remain one of the most important tools for diagnosing these entities.

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Key words: erythroleukemia, pure erythroid leukemia, classification, peripheral blood and bone marrow morphology, pinched red cells.

Erythroid leukemia is a very infrequent type of acute leukemia (2-5%). Most authors agree that two main subtypes exist: the M6 acute leukemia according to the FAB criteria in which mixed granulocytic and erythroblastic cellular
components are present; there also exists a pure erythroid acute proliferation not taken into consideration in the FAB classification. The recent WHO classification of acute erythroid leukemias recognizes two subtypes based on the presence or absence of a significant myeloid component: 1) erythroleukemia (erythroid/myeloid) with 20% or greater of myeloblasts and 2) pure erythroid with more than 80% of the marrow cells being erythroid with no evidence of a significant myeloblastic component (<3%) and usually <10% maturing erythroblasts. A clear-cut separation between these entities is not always possible and in most patients there is a gradual progression from one type to the other. Sometimes it is a continuum that spans a myelodysplastic syndrome with erythroid dysplasia and ineffective erythropoiesis and a mixed erythroblast and myeloblastic proliferation eventually ending in an acute myeloblastic leukemia if the patient survives long enough.

Although the new genetic technology is an important support to conventional morphology permitting better characterization of the different types of acute leukemias, acute erythroid leukemias do not yet have a specific genetic marker that allows them to be identified. This fact encouraged us to pay special attention to the morphology of these rare entities complemented by some biological features. The main aim of our cooperative study based on 62 patients was better delineation of these entities based on morphology, especially that of erythroblasts and erythrocytes.

Design and Methods

Sixty-two patients with acute erythroid proliferations were studied. The clinical features of all cases were reviewed with particular attention given to previous medical history, including exposure to toxic agents, chemotherapy and radiotherapy treatment and presence of autoimmune diseases. Age, sex and numerical findings of peripheral blood, bone marrow, and cytochemical stains (iron stores, PAS reaction, myeloperoxidase and unspecific esterases) and phenotypic markers (CD34, CD117, CD13, CD33, CD15, anti-MPO). The erythroid nature of the blast cells belonging to this series was confirmed by ultrastructural studies and/or immunologic markers (CD45, CD36, glycoporphin-A, CD71).

Cytogenetic analyses of bone marrow samples were performed at the individual centers. Whenever possible, at least 20 metaphases were analyzed and 10 of them karyotyped. Chromosome identification and karyotyping followed the International System for Chromosome Nomenclature (ISCN, 1985; 1995).

Clinical outcome was collected from the medical records; as the treatment was not uniform and depended on consultants’ criteria at the different institutions, we could not draw any conclusions.

Results

There were 35 male patients and 27 female ones. Their median age was 67 years (range 13-94).

Clinical findings. In our series 53 out of 62 patients (85.5%) met the criteria for M6a leukemia of the WHO classification and 2/62 (3.2%) were classified as having pure erythremia according to the WHO classification. Thus, in our study 7/62 patients (11.1%) remained for whom we had conclusions.

Fifty-five patients presented with de novo acute leukemia (sudden onset, without previous radiochemotherapy, toxic exposure or neoplastic or autoimmune diseases). In three of the remaining patients a myelodysplastic syndrome preceded the AML (13 months, 4 and 13 years previously); in two
patients the disease was therapy-related, one patient had undergone kidney transplantation ten years previously and one patient had been chronically exposed to solvents.

Laboratory findings. Pancytopenia was common at presentation; anemia was significant with a median hemoglobin 78 g/L (range 6-110) and mean cell volume (MCV) 96 fL (range 71-118); low leukocyte counts were common with median white blood cells of 2.7×10^9/L (range 0.7-26). Thrombocytopenia was frequent with median platelets of 42.0×10^9/L (range 3-512). Circulating blast cells were noted in 8% (range 0-72) of peripheral blood smears.

Morphologic findings in peripheral blood

The most frequent erythrocytic dysplastic features present in blood smears were schistocytes in 36 out of 50 (72%), tear-drop forms in 37 out of 50 (74%), pincered red cells in 33 out of 51 (60%), and basophilic stippling in 22 out of 50 (44%). Other abnormalities of red cells such as Cabot rings, and Howell-Jolly bodies were rare (<10%). Circulating erythroblasts were observed in 26 out of 51 (51%) cases. The following leukocyte dysplastic features were found in blood smears: hypogranulation in 18 out of 46 (39%), hyposegmentation in 17 out of 44 (39%) and abnormal chromatin clumping in 2 out of 41 (5%).

Platelet dysplastic features in blood smears were: giant platelets in 24 out of 44 (55%), hypogranulation in 16 out of 41 (39%), and micromegakaryocytes in 4 out of 40 (10%).

Bone marrow findings

Bone marrow was hypoplastic in two cases and the remaining sixty patients showed normal or increased cellularity. There were megaloblastic changes in 53 out of 62 (85%), multinuclearity in 34 out of 52 (65%), karyorrhexis in 33 out of 62 (53%), basophilic stippling in 31 out of 62 (50%), basophilic stippling and abnormal distribution of hemoglobin in 22 of 62 (35%), morulae in 16 out of 62 (25%), cytoplasmatic vacuolization in 13 out of 56 (23%), and internuclear bridges in 10 out of 60 (17%) (Figure 1).

Cytochemistry

The PAS reaction displayed a coarse positive pattern in immature erythroblasts in 8 out of 51 (16%) cases, and showed a diffuse pattern in 29 out of 51 intermediate and mature erythroblasts (57%). An increase of bone marrow iron (Perl’s reaction) was a common feature, and in 19 out of 44 (43%) patients ring sideroblasts were detected; in 34% of these patients they had been suspected applying only Giemsa stain. The granulocytic series showed hypogranulation in 42 out of 62 (68%) cases, giantism in 20 out of 62 (32%), hyposegmentation in 16 out of 62 (26%) and myeloblasts with Auer rods in 9 out of 62 (15%). An increase of histiocytic cells with phagocytosis was observed in 13 out of 62 (21%) patients. Megakaryocytic lineage was frequently involved with micromegakaryocytes in 25 out of 57 (44%), unconnected nuclei in 23 out of 53 (43%), and mononuclear elements in 12 out of 57 (21%).

Trilineage dysplasia was present in 36 of 62 patients (54%). In 7 patients the only dysplastic finding in the erythroid cells was megaloblastosis. In 16 patients (26%) the granulocytic cells did not show dysplastic features.

Cytogenetic results

In 30 out of 62 patients a cytogenetic study was available. Karyotypes were normal in 14 out of 30 cases. The remaining 16 patients showed an abnormal karyotype. Complex karyotypes with multiple numerical/structural abnormalities were detected in 10/16 patients. Involvement of chromosome 5, 7 or 8, in addition to another abnormality was present in 5, 3 and 6 cases, respectively.

Survival data

We have survival data for 45 patients. Eighteen out of these 45 patients died in the first six months following diagnosis, 13/45 died between six and twelve months, 6/45 survived less than twenty-four months and 8/45 are still alive (range: 2-8 years). Most long time survivors had undergone a
bone marrow transplantation.

Table 1 lists the characteristics of the seven patients for whom we had classification problems. Two patients (JCV, LCP) presented with 40% and 42% of erythroid blasts, with a total erythroblast count of 91% and 63%, respectively, without myeloblasts. In consequence the total percentage of the erythroblastic series fitted the diagnosis of pure erythremia but a considerable number of erythroblasts had some degree of maturation, even reaching the orthochromatic stage. The patient with a total erythroblast count of 63% showed PAS positivity with a coarse granular pattern and a complex karyotype. Two of them, patients JRG and AVP had, respectively, 20 and 25 ringed sideroblasts and fitted criteria for the diagnosis of refractory anemia with ringed sideroblasts in the FAB and WHO classifications; however, the degree of erythroid infiltration (67 and 79%), severe dyserythropoiesis, a coarse PAS reaction, and very poor survival are very uncommon in refractory anemias with ringed sideroblasts and in our opinion these features fit better the old concept of acute erythremia with maturation. Three patients had a very important degree of erythroblastic infiltration (80, 90 and 66%). Neither myeloblasts nor ring sideroblasts were observed. Severe dyserythropoietic changes and a coarse PAS reaction were present; taking all findings together, the old diagnosis of erythremic myelosis might be applied.

Surprisingly only a few articles have paid particular attention to erythrocytic morphology in erythroblastic proliferations. Schistocytes and teardrop cells were outstanding features.11,12 Our findings are in concordance with these, adding the conspicuous presence of the so-called pincered cells not described before in this pathologic context. We want to emphasize that pincered cells were found in 60% of our patients and might indicate an involvement of the band 3 transmembrane protein. A defect of band 3 was found in some cases of hereditary spherocytosis in which, in addition to the presence of spherocytes, some erythrocytes showed the morphologic alteration called pincered red cells, mimicking the shape of a mushroom (Figure 2).13

We compared the incidence of these abnormal erythrocytes in M6 (60%) with that in other acute myeloid leukemias (13%) and the difference was statistically significant (p < 0.0001). Unexpectedly, in four patients we observed the presence of circulating micromegakaryocytes in the peripheral blood. This finding was not correlated with other characteristics, such as features of myeloproliferation or a special or outstanding dysplasia of megakaryocytes.

In bone marrow the morphology of the ery-
throphoblasts was not particularly impressive and indeed 10% of the patients had no morphologic anomalies or only minimal ones. Very non-specific megaloblastic changes were frequently observed and were present as a sole anomaly in about 10% of all our patients. These patients with minimal or even absent dysplastic red cells or their precursors did not correlate with those with the presence or absence of abnormal erythrocytes such as pincered cells. Cytochemically PAS positivity is a hallmark of pathologic erythropoiesis and was detected in 73% of our cases; positivity was coarse in the most immature erythroblasts and diffuse in more mature elements. Abnormal iron deposits with a few ring sideroblasts were found in nearly half of our patients; in many cases this finding could be anticipated by the peculiar aspect of the erythroblast stained with May-Grünwald-Giemsa.

Regarding other myeloid lineages, dysplastic features were non-specific. The megakaryocytic lineage was the second most affected cellular line, with dysplastic features such as micronuclear elements and megakaryocytes with multiple unconnected nuclei. Involvement of the megakaryocytic series is not surprising if we remember the close links, at least in some species, between erythroid (BFU-E) and megakaryocytic (MegFU) precursors. The granulocytic series was the least affected when involved.

No specific chromosome abnormality has been described in acute myeloid leukemias with a predominant erythroid population. As regards chromosomal aberrations, complex karyotypes were observed in over half the cases. Chromosomes 5, 7 and 8 were the most frequently affected, concordant with previously published data; they never presented as a sole abnormality but frequently in a complex karyotype. As expected secondary acute erythroid/myeloid leukemias more often showed an abnormal karyotype than de novo acute leukemias.

As expected most of these patients had a short survival; but surprisingly 7% of this series had a long survival, probably related to aggressive chemotherapy followed by allogenic bone marrow transplantation in young patients.

Acute erythroid malignant proliferations encompass a wide spectrum of rare diseases among which the erythroid/myeloid leukemia of the WHO classification is the most frequently observed. According to the new instructions of the WHO classification, the 54% of our cases which showed trilineage dysplasia should be dropped out from the group of acute myeloid leukemia not otherwise categorized and reclassified as acute myeloid leukemia with multilineage dysplasia. In these diseases with a wide biological spectrum, despite improvement of ancillary tests, morphologic characterization remains an important diagnostic tool.

Contributions and Acknowledgments

All the authors contributed to the conception of this study and to the collection of data. AD-C was primarily responsible for this work. IL and AD-C analyzed and interpreted the data, and made the literature search. FS and AV performed the cytogenetic studies. AD-C and SW drafted the article and its revisions. Order of authorship: authors are listed according to the extent of their contribution to the work, while the last author had a major role as senior author in the conception and revision of the article. All the authors approved the submitted version of the article.

Disclosures

Conflict of interest: none.
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References

Acute erythroid neoplastic proliferations


Peer Review Outcomes

What is already known on this topic
Since the pioneer studies by Giovanni Di Guglielmo there has been no further break-through in our understanding of erythroid neoplastic proliferations. Most hematologists associate this latter definition with AML-M6, and perhaps with refractory anemias.

What this study adds
This study shows that, while the optical microscope remains an important tool for diagnosing erythroid malignancies, a portion of them do not fit neatly into any of the WHO classification categories. Immunophenotyping and molecular investigations will likely provide a deeper insight into these mysterious conditions that fascinated Di Guglielmo several decades ago.

Manuscript processing
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Potential implications for clinical practice
Facing a patient with a potential erythroid malignancy, full morphologic and immunocytochemical studies should be performed for a proper diagnosis. In fact, the erythroid disorders to be included in the differential diagnosis process, may span from a benign condition such as refractory anemia with ring sideroblasts to aggressive erythroleukemia.

Mario Cazzola, Editor-in-Chief