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Minimally differentiated acute myeloid leukemia (AML-M0): a distinct clinico-biologic entity with poor prognosis

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Abstract FAB proposals for the diagnosis of AML-M0 represent the formal recognition of a distinct entity which has been described over the past few years by several authors and called minimally differentiated acute myeloid leukemia. By definition, AML-M0 includes acute leukemias which do not fit morphological and cytochemical criteria for the diagnosis of AML, and for which myeloid lineage assignment can be made by immunological assay showing positivity for MPO, CD13, and CD33 and negativity for lymphoid markers. Involvement of an early myeloid progenitor in the leukemic process is a possible theory hypothesized to explain the existence of such a form. Validity of this assumption has been based on the observation that AML-M0 frequently bears "stem cell" markers such as CD34, HLA-DR, Tdt, CD7, and promiscuous IgH/TCR gene rearrangements, which are thought to occur in uncommitted cells. Finally, AML-M0 very frequently carries cytogenetic abnormalities common to MDS or secondary AML, such as -5/5q- or -7/7q- deletions and or complex karyotype. In our experience, AML-M0 is also very often associated with the MDR phenotype, which in turn has been found strictly linked to "stem cell" features, especially in MDS. These biological aspects, altogether, translate into a very unfavorable prognosis, confirming even from a clinical point of view that AML-M0 is a distinct entity. In conclusion, "stem cell" markers, MDR phenotype, complex chromosome lesions, frequent occurrence in elderly patients, and intrinsic chemoresistance characterize AML-M0 and indicate the need for tailored treatments, possibly involving the use of MDR modulators and/or differentiating agents.

Key words AML-M0 · Anti-MPO · Complex karyotypes · MDR phenotype

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Introduction

In 1976, and subsequently in 1985 the French-American-British Cooperative Group (FAB) defined standardised criteria to qualify acute leukemias as myeloid (AML) or lymphoid (ALL) [1, 2]. Distinction between AML and ALL is basically made on morphological and cytochemical grounds; in this view, the FAB group proposes that AL with fewer than 3% myeloperoxidase (MPO) and/or sudan black B (SBB)-positive blasts should be considered ALL, whereas those with greater than 3% MPO and/or SBB-positive blasts are AML. However, over the past few years, the increasing use of monoclonal antibodies (moabs) as a diagnostic tool has led to the identification of some cases which do not fit the FAB criteria for AML, but rather those for ALL. Using ultrastructural cytochemistry and myeloid immunological markers, several groups demonstrated that a minority of the cases with $<3\%$ MPO/SBB-positive blasts, which would previously have been classified as ALL, in fact showed myeloid maturation; these cases have been named "minimally differentiated" AML (AML-M0) [3–9]. Accordingly, in 1991, the FAB group put forward the criteria for the recognition of this new entity: the diagnosis of AML-M0 is made if less than 3% of the blasts are MPO/SBB positive, if the blasts stain for myeloid-associated markers, and if they are negative for B/T-lineage antigens [10]. At present, AML-M0, along with AML-M7, represents the only exception to the FAB scheme where immunological markers are included as a part of diagnostic criteria. In this review we focus on the biological features and clinical outcome of AML-M0.

Biological features

Morphology and cytochemistry

Bone marrows from AML-M0 cases are typically hypercellular with light microscopical blast appearance often resembling ALL-L2. Blasts are typically very large with rounded nuclei, open chromatin, and prominent nucleoli. The cytoplasm is usually scant and moderately basophilic, while heterogeneity of the nuclear shape is observed in some cases. Azurophil granules or Auer rods are not seen in the blast cells, whereas some evidence of dysmyelopoiesis affecting any of the three lineages can be present [3, 6–12]. By cytochemistry none or $<3\%$ MPO/SBB-positive blasts are observed, thus excluding the diagnosis of AML-M1 (low percentage of MPO/SBB positive blasts) [10]. Alpha-naphthyl acetate esterase staining can be positive in some cases and the reaction remains positive after inhibition with sodium fluoride [6, 10]; some authors also described faint alpha-naphthyl butyrate esterase and PAS positivity [6]. Our group has also observed, in some instances, positive staining for acid phosphatase. The reaction is characterized by a diffuse, granular pattern of positivity (unpublished observation).

Ultrastructural cytochemistry

The method involving the use of electron microscopy (EM) is intended for ultrastructural demonstration of small primary granules containing MPO in very early myeloid cells like those found in AML-M0 [8, 10]. In a comparative analysis, Shetty et al. [13] evaluated the presence of MPO in leukemic blasts by light microscopy (LM), immunocytochemistry (IM), and EM. The authors investigated 14 cases of AL; six were recategorized as AML based on ultrastructural MPO positivity established by the immunogold technique. All of them were MPO negative by LM, while three were MPO positive by IM. The investigators concluded that EM is more sensitive for showing MPO than LM and IM.

Immunophenotyping

Myeloid marker expression

Immunological studies are needed in order to establish the myeloid origin of blasts which are, by definition, morphologically and cytochemically undifferentiated. Reactivity with moabs against myeloid-associated antigens, usually CD13 and CD33, is the key finding to qualify AL as AML-M0. Bennett et al. [10] reported the expression of CD13 and CD33 in 70% and 80% of AML-M0 cases, respectively, and in all of them at least one myeloid marker was clearly positive. Lee et al. [3] observed myeloid features (CD13 and CD33 positivity) with all samples having at least one myeloid antigen expressed on the membrane of the blasts. In their description of 14 patients with AML-M0, Segeren et al. found that CD33 and CD13 were expressed in 100% and 46% of cases, respectively [9]. Cuneo et al. [11] detected positivity for two or more myeloid markers in 22/26 cases and in only two cases was either CD13 or CD33 positive in $>20\%$ of blast cells. In our experience [6, 7, 12] CD13 and CD33 were expressed in 70% and 65% of the cases, respectively; in 50% of the samples they were co-expressed, while in 13% both were negative. One of these patients carried CD15 as the only myeloid marker. The recognition of AML-M0 is also established with methods that include detection of MPO in blast cells by immunological techniques [6, 7, 10, 14, 15]. MPO is localized in the primary granules of myeloid cells, and its synthesis occurs early on the differentiation pathway [14, 16, 17]; it therefore is a good candidate as a specific immunological target for AML. A study involving 140 patients affected by AL (90 AML, 50 ALL) has confirmed the sensitivity and specificity of anti-MPO when used in immunocytochemistry with the APAAP method [8]. Van der Schoot et al. [14] reported the results of immunophenotyping blasts of 206 patients with AML with a moab anti-MPO. Compared with other myeloid markers, a greater diagnostic sensitivity and specificity was documented in favor of anti-MPO. In our experience, 100% of 23 patients affected by AML-M0 showed anti-MPO positivity as compared with CD13 and CD33, which were both not expressed in 13% of the cases [6, 12]. In the study of Praxedes et al. [16], based on the anti-MPO positivity, five out of ten leukemias called undifferentiated (AUL) were reclassified as AML-M0, though four of them were CD13/CD33 negative. The authors concluded that anti-MPO is a very sensitive and reliable tool in AML diagnosis and has a pivotal role in distinguishing AML-M0 and byphenotypic acute leukemia from AUL and ALL. During the diagnostic procedures for 750 AL cases, Campana et al. [17] identified nine cases which were morphologically, cytochemically, and phenotypically undifferentiated. In one of these, 7% of blasts were labeled by the anti-MPO, thus suggesting the presence of peroxidase in its proenzyme form. Although Pombo de Oliveira et al. [18] emphasized that the myeloid marker CD13 is detected in the cytoplasm of myeloblasts earlier than on the membrane, and should therefore be tested by immunocytochemistry whenever negative on cell suspension, MPO is likely to be the most specific marker of myeloid lineage. Moreover, application of immunological techniques to MPO determination has also practical implications, as CD13/CD33 can be inappropriately expressed in some cases of ALL, and EM is not always available in many laboratories.

Stem cell marker expression

The expression of immaturity markers occurs frequently in AML-M0. Cuneo et al. observed CD34 positivity

in all the cases examined, associated with a consistent expression of HLA-DR [11]. In the experience of Lee et al., CD34 and HLA-DR were positive in 50% and 100% of cases, respectively [3]. Segeren et al. reported a high frequency of expression of these markers; in fact, CD34 and HLA-DR stained positive in 100% and 61% of cases, respectively [9]. Ninety-six percent of our own cases were CD34 positive, while HLA-DR was expressed in 91% of them [12]. Markers such as terminal deoxynucleotidyl transferase (Tdt) and CD7 deserve special mention, as they are associated with either ALL or immature myeloid leukemias [19–23]. Tdt is a nuclear enzyme that is expressed both in normal and in leukemic B/T cells and is involved in promotion of immunoglobulin/T-cell-receptor diversity by randomly adding nucleotides to the N region during heavy chain gene assembly [24, 25]. It has become evident, however, that although Tdt is preferentially found in ALL it is also detected in a variable proportion of myeloid leukemias [19, 26–31]. Whether the positivity of Tdt in AML represents the engagement of a multipotent stem cell retaining multiple differentiation antigens or reflects a misprogramming of the differentiation pathway leading to "lineage infidelity" remains to be determined. As a matter of fact, some authors have reported a significantly higher incidence of Tdt in poorly differentiated myeloid leukemia such as AML-M0/M1 [6, 7, 9, 12, 19, 31, 32]. Bennett et al. [10] found Tdt to be positive in one of four AML-M0 cases evaluated. Lee et al. [3] reported that 2/10 patients with AML-M0 had Tdt detected in a minority of their blasts. Segeren et al. [9] documented Tdt positivity in 73% of their cases. Cuneo et al.[11] observed inappropriate expression of Tdt in seven (27%) of 26 cases of AML-M0. Finally, in our analysis Tdt tested positive in 65% of the samples [6, 7, 12]. Moabs recognizing the CD7 moiety identify a 40-kD protein expressed on the membrane of peripheral T cells, thymocytes, and T-derived leukemic blasts [33–38]. The expression of CD7 occurs early during T-cell ontogeny, before the appearance of other T markers and prior to T-cell-receptor β-chain gene rearrangement [34]. In this view, the recognition of CD7 expression has proved particularly effective in the diagnosis of ALL of T origin [35–38]. On the other hand, the specificity of CD7 has been debated, since its widespread evaluation brought about the existence of CD7+ AML [39–45]. In addition, Chabannon et al. [46] documented the presence in normal bone marrow of a subset of CD34/CD7-positive cells with myeloid potential. The authors concluded that CD7 is not restricted to T cells, but is also expressed during early stages of myeloid differentiation. Thus, the presence of this antigen on myeloblasts might suggest origin from immature precursors [20–22]. Cuneo et al. [11] found that CD7 was expressed in 14 (54%) of 26 cases of AML-M0; Segeren et al [9] have reported a frequency of CD7 positivity of 43% in their analysis; CD7 was positive in 30% of our AML-M0 cases [12].Altogether, these observations consistently support the hypothesis of CD7 association with "stem cell" derived leukemia and with the expression of immaturity markers such as CD34 and Tdt.

Lymphoid markers expression

Negativity for lymphoid antigens is one of the major criteria mentioned in the FAB classification for the diagnosis of AML-M0 (isolated expression of Tdt or CD7 does not preclude the diagnosis of AML-M0) [10]. We investigated 23 patients with AML-M0, and detected CD2, CD10, and CD19 in three (13%), two (9%) and one (4%) of the cases, respectively. The concurrent expression of lymphoid markers was observed in five cases (one Tdt+CD19, one CD7+CD2, one CD7+CD10, one CD7+CD2+Tdt, and one CD2+CD10+Tdt) [6, 12]. In spite of the presence of two or three lymphoid antigens, we felt these cases should be regarded as AML-M0 with "minimal phenotypic deviation" rather than as biphenotypic leukemia, based on the cCD3/cCD22 negativity and the low score for biphenotypic leukemia proposed by Buccheri et al. [47]. Providing MPO positivity, by immunoassay or EM, we believe the expression of lymphoid markers is not necessarily against a diagnosis of AML-M0.

MDR protein expression

We also investigated the expression of P-170 glycoprotein in our AML-M0 cases, and it tested positive in 40% of the samples evaluated (6/15) [6, 7, 12]. This finding is in agreement with previous reports indicating that P-glycoprotein expression is tightly linked to a stem-cell phenotype [48, 49] and also explains the chemoresistance of these forms, providing a rationale for the use of revertant agents.

Cytogenetics

It is well established that AL is often characterized by specific or recurrent chromosomal lesions which, in all probability, underlie the genesis of the disease and define selected subsets with different prognosis and clinical outcome. Several groups have tried to investigate the cytogenetic features of AML-M0, with controversial results [3, 6, 7, 9, 11, 12, 50–54]. Cuneo et al. [11] found a higher incidence of abnormal/complex karyotypes and unbalanced chromosome changes (81%) in AML-M0 as compared with a reference sample including AML-M1. The authors stated that AML-M0 has a specific cytogenetic profile, often recalling that of myelodysplasia and secondary AML, which may account for the unfavorable outcome of these leukemias. In addition, the frequent detection of -5/5q- and -7/7q- deletions or translocations involving 12p, which are recurrent findings in erythroleukemia, support the assumption that some cases of AML-M0 may arise as part of

immature erythroid proliferation. Trisomy 13 was also observed in five patients – in two as the sole anomaly, in three associated with additional changes. A significant association between trisomy 13 and "stem cell"-derived leukemia has been described previously [55, 56]; furthermore, it has been pointed out that this anomaly may select a subset of AML-M0 especially affecting elderly males with a history of professional exposure to toxic agents [11]. Segeren et al. [9] reported an incidence of chromosome aberrations in 58% of their cases and, although three samples bore the 7q deletion, no consistent or specific lesions were found. Among six cases of AML-M0, Yokose et al. [54] observed one complex karyotype (47,XY, +18/47XY,+18,7q-), while the remainder were normal. Lee et al. [3] described the occurrence of chromosome abnormalities in 87% of the samples examined and, in agreement with the observations of Cuneo et al., they found a predominance of complex karyotypes with involvement of chromosome 7 in three cases and chromosome 5 in one case. Nineteen of 23 of our patients were subjected to cytogenetic analysis, and 15 (79%) were found to carry aberrations [6, 7, 12, 50]. Lesions were generally complex (42%) and in 26% of the cases -5/5q- and/or -7/7q- deletions were recognized. Trisomy 8, 4, and 13 were observed in two, two and one case, respectively. The occurrence in one patient of the t(6;11)(q15;q23), which is strongly associated with monoblastic/monocytic leukemia, prompted the question of whether, under certain conditions, AML-M0 may be part of monocytic proliferation. In fact, the patient eventually relapsed as an M4/M5 form. According to the recent data available, AML-M0 stands out as a distinct entity with a high incidence of abnormal karyotypes such as complex lesions, deletion -7/7q- and/or -5/5q-, and trisomy 8, 4, and 13, which may partially explain its inferior prognosis.

Molecular biology

Few data have been generated concerning molecular features of AML-M0; as a consequence, only limited information can be extracted from the literature. Campana et al. [17] demonstrated a germ-line configuration of IgH and TCR$\beta/\gamma/\delta$ in the unique case of AML-M0 described among nine AUL cases. Yokose et al. [54] investigated IgH/TCR molecular rearrangements in two of five AML-M0 and in both germ-line status was detected. Cuneo et al. [11] have reported the more numerically consistent series as regards IgH and TCR molecular studies in AML-M0. Rearrangements of IgH and TCR$\beta/\gamma/\delta$ were looked for in 15/26 cases of AML-M0. The authors identified IgH, TCRγ, and δ rearrangement in four, three, and two cases, respectively. In two cases concurrent rearrangement of IgH and TCR were observed (one IgH+TCRγ, one IgH+TCRγ+TCRδ). There were no rearrangements of TCRβ. No firm conclusions could be drawn on the biological meaning of these findings, and the authors concluded that the incidence of inappropriate rearrangements of IgH and TCR in their series was, after all, not dissimilar to that of previous studies including unselected cases of AML [57, 58]. However, the occurrence of inappropriate IgH/TCR rearrangements in AML-M0 is a paradigmatic example of "lineage promiscuity". This event has frequently been discovered in striking association with immature acute leukemia and has been interpreted as aberrant genetic disease-related programming [59, 60]. Conversely, other authors [61] suggest that lineage infidelity may not imply genetic misprogramming, but rather may reflect the existence of a transient phase of gene expression promiscuity, physiologically occurring even in normal precursors. Any factor causing maturational arrest induces the emergence of cellular clones bearing the "promiscuity marker" as a relic. A possible explanation offered for this theory is that, owing to recombinase activity, DJ rearrangement can occur in uncommitted cells and operates as a necessary but insufficient genetic step in lymphoid commitment. Subsequent successful DJV recombination irreversibly determines cell destiny toward the B or T pathway. Failure in adequate DJV rearrangement would permit alternative lineage switches to be decided (included myeloid switch). Based on these considerations, the association of promiscuous IgH/TCR rearrangement with AML-M0 cannot be of general significance but certainly reflects the involvement of an immature precursor, as also confirmed by the frequent expression of CD34, Tdt, CD7, and HLA-DR [62].

Treatment outcome

Although reports of treatment of AML-M0 are limited and optimal therapy has not yet been established, it is generally accepted that poor results are obtained with conventional chemotherapy. Lee et al. [3] reported a complete remission (CR) rate of 20% (2/10) with standard chemotherapy: in one patient CR lasted 5 months and the remaining patient died of therapy-related complications while in CR. Median age of the population was 52 years (range 34–71), median WBC was 4.75×10^9/L (range 0.6–175); with regard to sex, the group was well balanced, with five men and five women. In the experience of Segeren et al. [9], 4/14 (29%) patients entered CR, but only one is in continuous CR (17 months), while the other three relapsed with a median duration of CR of 4.5 months; no second remissions were obtained. The median survival of the entire group was 4.5 months (range 1.5–32). The median age of the patients was 62 years (range 7–77); eight were men and six women, and median WBC was 5.45×10^9/L (range 0.7–78.6). Cuneo et al. [11] observed a CR rate of 54% among 26 patients whose median age was 60 years (range 15–79) and WBC was 4.9×10^9/L (range 1.2–64). The median duration of CR and survival was 6

and 8 months, respectively. We obtained a CR rate of 22% (5 patients) among 23 patients treated with conventional therapy [7, 12]. The median duration of survival and CR was 2 and 3 months, respectively (Figs. 1 and 2). Four patients relapsed, and salvage treatments failed to induce second remissions; one patient died in CR of fungal infection. Our patients had a median age of 60 years (range 27–81) and a median WBC of 7.2×10^9/L (2.3–173); distribution according to sex was homogeneous (12 men and 11 women). Yokose et al. [54] described CR in 3/5 patients (60%) with AML-M0; in one case CR lasted only 5 days. Of the four patients described by Foon et al.[63], three achieved CR, but no data on survival are available. Mertelsmann et al. [64]

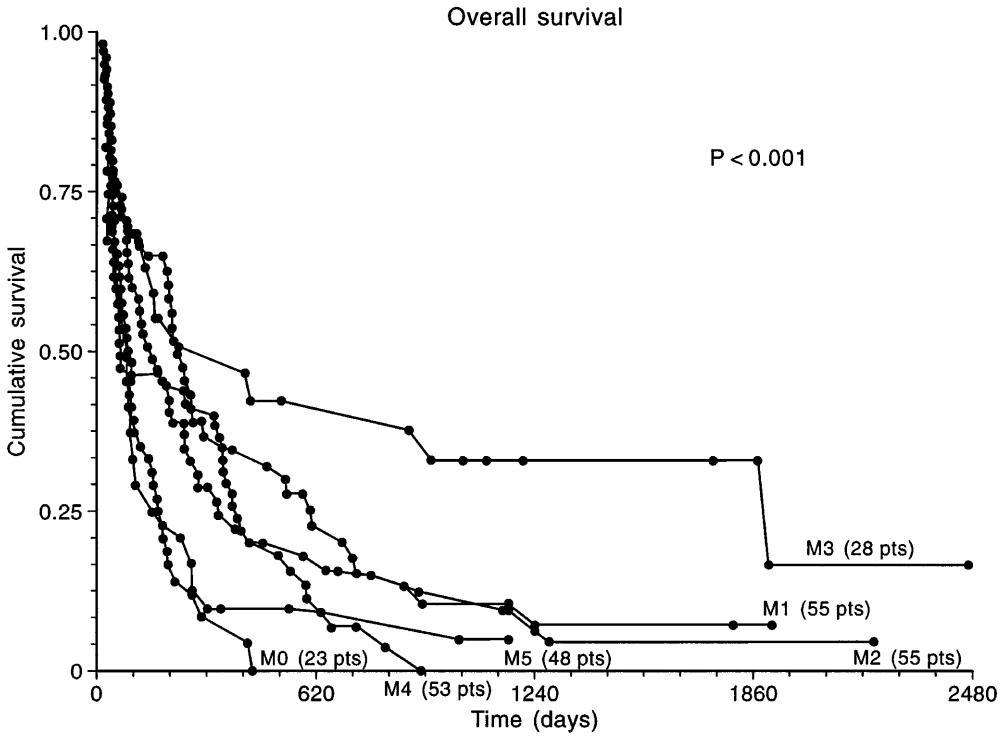

Fig. 1 Overall survival according to FAB subtypes, for 264 patients (pts) seen at our institution from 1987 to 1995

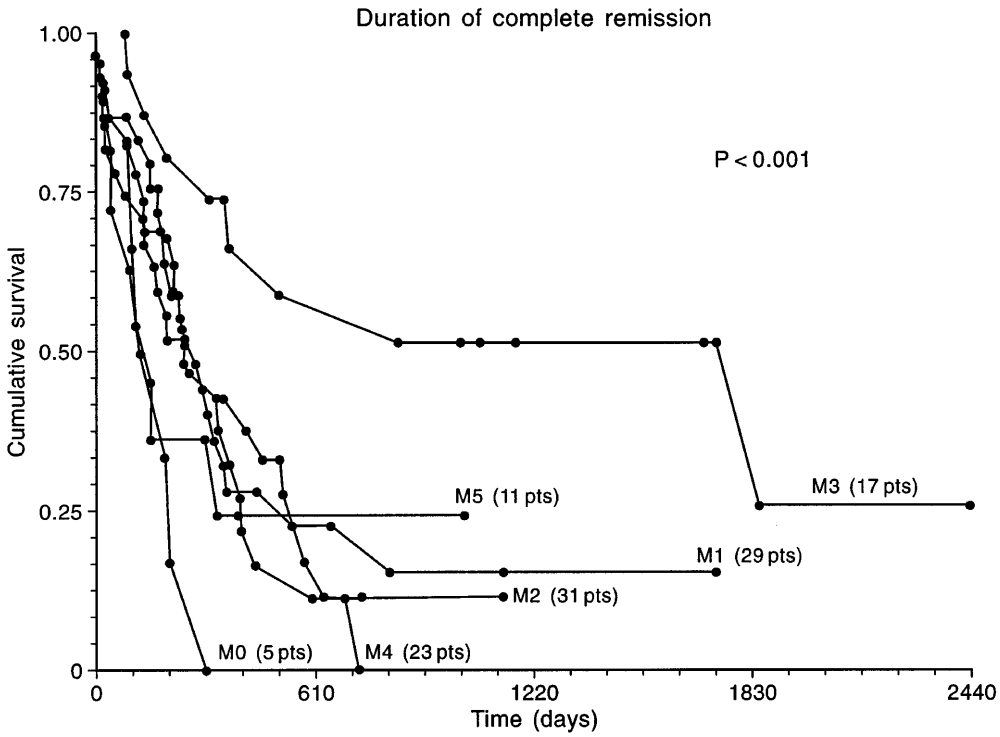

Fig. 2 Duration of complete remission according to FAB subtypes, for 264 patients (pts) seen at our institution from 1987 to 1995

noted either a decrease in CR or a shortened duration of survival and CR in a mixed group classified as AUL. In revising their results in patients with AML-M0, Reiffers and Broustet [65] noted that all five cases which were MPO positive by EM failed to enter CR. This whole body of evidence confirms, even from a clinical point of view, that AML-M0 is indeed a distinct subtype of AML with a disappointing response to conventional treatment.

In conclusion, AML-M0 is a recently established entity whose diagnosis derives from an integrated approach including morphology, cytochemistry, immunophenotyping, cytogenetics, and EM. Based on the data from the literature as well as on our own experience, we can conclude that: (a) AML-M0 presumably originates from a very early committed granulocytic progenitor, as suggested by the expression of CD34, Tdt, CD7, and HLADR and by the occurrence of inappropriate IgH/TCR recombination. (b) Anti-MPO is a sensitive reagent to recognize AML-M0; it is perhaps more useful than CD13/CD33, which are reported to sometimes be negative, while MPO is always expressed (by immunoassay or EM). (c) In our opinion, the expression of lymphoid markers does not exclude a diagnosis of AML-M0, as anti-MPO, CD13, CD33 positivity and cCD3/cCD22 negativity are major criteria for myeloid lineage assignment. (d) P-170 MDR protein is frequently expressed in AML-M0. (e) From a cytogenetic point of view, AML-M0 is characterized by a high incidence of complex and/or unbalanced chromosomal aberrations, with a frequent recognition of -7/7q- or -5/5q- deletions often resembling MDS and secondary AML. (f) From a clinical point of view, AML-M0 develops especially in elderly patients (≥ 60 years). The convergence of the above-mentioned factors accounts for the very unfavorable prognosis of AML-M0 and for the poor response to standard therapy. In the light of these findings, the necessity of rendering chemotherapy more intensive has been put forward, but only a minority of patients will be able to afford intensified treatment, considering that most of them belong to older age-groups. Under these circumstances, the use of alternative treatments incorporating MDR modulators and/or differentiating agents is advisable.

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