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Acute myeloid leukemia and related conditions

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The medical community's understanding of acute myeloid leukemia (AML), what it is and which criteria to use to establish the diagnosis, has been in sporadic evolution for more than 130 years. This article discusses acute myeloid leukemias, myelodysplastic syndromes, and those disorders manifesting features of myelodysplasia and myeloproliferative diseases as defined by the World Health Organization (WHO) in the monograph *Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues* [1] published in 2001. As our armamentarium of diagnostic tools has increased, we have gone from classifications of acute leukemia based purely on patient survival to divisions by morphology with the aid of cytochemistry to the present, whereby entities are defined by immunologic markers, histochemical stains, genetic alterations, and morphology. The focus here is on the diagnosis of acute myeloid leukemia and those disorders leading to AML as aided and defined by flow cytometry. There is little disagreement that flow cytometry is the best available method of immunophenotyping acute leukemias with current technology. Hematology, however, is not ready to dispense with our old friend, the light microscope.

Although the diagnosis of acute leukemia is more than a century old, there were no set criteria for the diagnosis until the advent of the French-American-British Cooperative Study Group (FAB) [2]. In the Armed Forces Institute of Pathology's first series fascicle *Tumors of the Hematopoietic System* by Henry Rappaport, MD, published in 1966, there were descriptions of the malignant cells in the various kinds of leukemias described but no mention of a minimum number of blasts for the diagnosis of acute leukemia. The FAB classification, first of acute leukemia (1976) [2] and then of myelodysplastic syndromes (1982) [3], was the first system to receive widespread acceptance, fostering a uniform classification of acute leukemia and myelodysplasia. It was hoped that a uniform and widely accepted classification system would reduce the subjectivity of a diagnosis of acute leukemia and allow for better comparison of new therapies.

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Even with FAB classification there were gray areas. With the availability of flow cytometry and the routine use of conventional cytogenetics, subgroups were identified that called for separation from this widely accepted classification. The WHO classification [1] used here revises the criteria for acute leukemia and the myelodysplastic syndromes while separating out chronic myelomonocytic leukemia and creating new divisions.

Flow cytometry and cell lineage

When one considers flow cytometry or immunohistochemistry for immunophenotyping leukemias and myelodysplastic syndromes, there is a tendency to consider most or all of the markers used as lineage specific. Most of the markers available are lineage-associated and not specific [4,5]. For separation of myeloid versus lymphoid, the markers that truly are lineage-specific, at least for the moment, are myeloperoxidase for myeloid, cytoplasmic CD3 (cyCD3) for T lymphocytes, and cyCD22 and cyCD79a for B-lymphocytes. CD14 and CD64 are the best markers to demonstrate monocytic differentiation [6]. Erythroid differentiation is indicated by anti-hemoglobin A and anti-glycophorin A [1]. CD61 and CD41 are the most commonly used markers for megakaryocytic differentiation [7]. Platelet membranes may adhere to blasts and other cells of interest. This can add difficulty for interpretation.

Other considerations include where to gate for analysis, choice of fluorochrome, and combination of antibodies used. Most laboratories use multicolor analysis for convenience and to obtain more information. Two-color flow cytometry may still be the norm, but three-color flow cytometry and even four-color flow cytometry are rapidly becoming the tools of choice for analysis of leukemias in the United States. Using CD45 and side scatter [8] to choose the region on which to gate has improved results over traditional forward scatter versus side scatter, aiding in excluding residual normal cell populations (Fig. 1). This technique may aid in detecting heterogeneous blast populations. All leukemias tend to have dim CD45 expression by comparison with normal lymphocytes and show greater side scatter than lymphocytes. Leukemias involving monoblasts and promonocytes tend to extend from the area typical of blasts into the area where monocytes are normally seen as a single population, as opposed to most acute leukemias of neutrophil precursors that tend to be a more discreet population separate from the maturing neutrophils. Some antigens such as myeloperoxidase (MPO) are cytoplasmic instead of residing on the cell surface, and others are lineage-restricted when detected in the cytoplasm, such as CD3. There are techniques to allow permeability of the cells to mark with these antibodies for the detection of antigens residing in the cytoplasm [9–11]. Immunohistochemical methods are superior for detection of MPO compared with flow cytometry [12,13]. For most other markers available for use by flow cytometry and immunohistochemical methods, flow cytometry is superior.

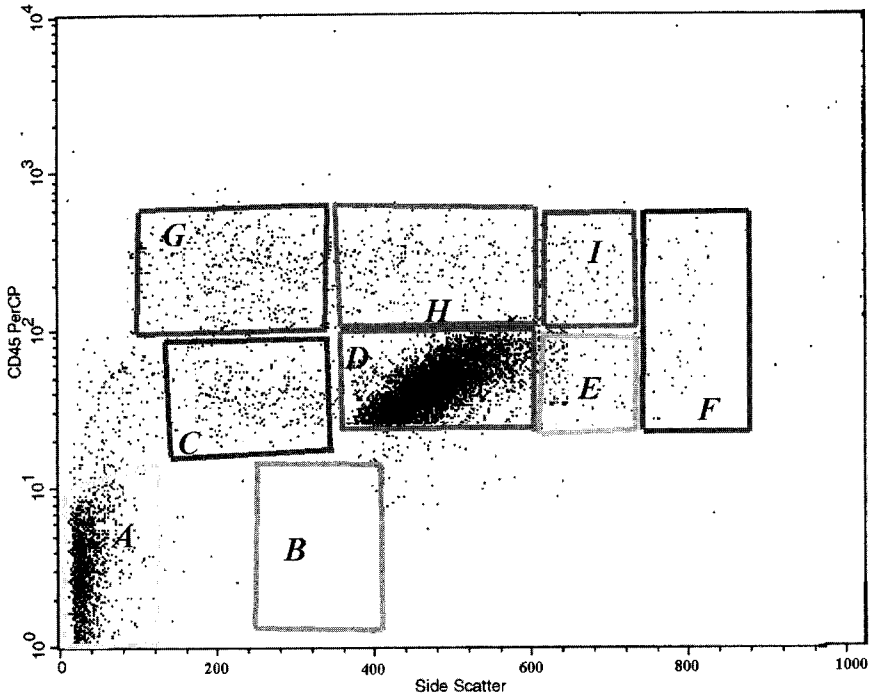

Fig. 1. Diagram of cell distribution with CD45 versus side scatter. A = debris; B = nucleated erythrocytes; C = lymphoblasts; D = myeloblasts and monoblasts; E = myelocytes and metamyelocytes; F = promyelocytes; G = lymphocytes; H = monocytes; I = segmented neutrophils.

There are markers that aid in separation of maturing cells from blasts of their own lineage. Acute leukemias, however, frequently do not follow the normal pattern of antigen expression. In normal megakaryocyte development, CD41 (glycoprotein IIb/IIIa) is a later marker than CD61 (glycoprotein IIIa). With normal monocytes CD11b, CD11c, CD 64, and CD68 are acquired later than CD33, CD13, and CD14, usually after the promonocyte stage. For neutrophil precursors and maturing forms, CD15 tends to be weakly expressed until after the myelocyte stage. Segmented neutrophils express CD10, CD66a, and CD66d. Eosinophils express greater density of CD15 as mature eosinophils, and express CD66b and CD66c. Mature basophils express CD9 and CD43 (not specific markers) and continue to express CD33, a marker usually lost on other types of mature granulocytes.

Acute myeloid leukemia

Acute myeloid leukemia is an uncontrolled proliferation of myeloid blasts. This process may be seen in the bone marrow, peripheral blood, and other tissues. The WHO classification [1] lowers the percentage of blasts from 30% to 20% for

the diagnosis. They also suggest that the percentage of blasts be determined by a 500 cell count on good quality, well-stained bone marrow aspirate slides and by a 200 cell differential on peripheral blood smears [1]. Flow cytometry can corroborate the manual differential in many cases. How to manage patients and if and when to treat them remains a clinical decision. For all of the acute leukemias involving neutrophil precursors, blasts mean myeloblasts except for acute promyelocytic leukemia wherein the abnormal promyelocytes are added to the myeloblasts for the diagnosis [1,2,14]. Monocytic processes include promonocytes with the monoblasts for diagnosing [1,14]. Erythroblasts are only key in the pure erythroid leukemia and are not counted per se, even in acute erythroleukemia [1]. CD34 and HLA-DR are markers for hematopoietic stem cells. These markers are present in many acute myeloid leukemias and in many cases of acute lymphoblastic leukemia (ALL). With the wide distribution of these markers, they are not considered lineage-specific. By no means do all blasts express CD34. Although terminal deoxynucleotidyl transferase (TdT) is more commonly expressed in ALL, it is not uncommon to detect TdT in many different varieties of AML [4,5,9]. Lymphoid-associated antigens such as CD7, CD19, CD10, CD3, CD5, and CD2 may be expressed in AML and myelodysplastic syndromes on the blasts involved by these disorders [15]. Bilineage leukemias will be discussed later but the presence of one or even two lymphoid-associated antigens is not sufficient to render such as diagnosis [1]. Even with the advances made in diagnostic capability, it is still necessary to obtain a bone marrow sample to judge pretreatment cellularity and to obtain the blast percentage if the blood displays pancytopenia or less than the required 20% blasts. In cases of hypocellular acute leukemia and those disorders associated with extensive fibrosis, the blast percentage may need to be determined from the bone marrow biopsy. Immunohistochemistry may aid in that determination [13]. There have been reports of AML in which the histochemical stains (MPO and Sudan Black B) showed reactivity but myeloid-associated antigens were not detected [16].

Acute myeloid leukemia not otherwise categorized

This grouping of AML is an updated version of the FAB classification [2], building on the revision by the FAB published in 1985 [14]. Most of the changes are evolutionary, not revolutionary. The numbering system is dropped in favor of the same named categories used by the FAB with some additions.

Acute myeloid leukemia, minimally differentiated

This category of AML is approximately the same as the FAB M0 AML [14]. The blasts typically express one or more antigens associated with myeloid differentiation such as CD33, CD13, or CD117 by flow cytometry [1,4,5,12]. There should be no evidence of lymphoid-restricted antigens (cyCD3, cyCD22, or cyCD79a). Occasional cases may express MPO by flow cytometry on a small subpopulation of blasts but many cases are negative for MPO. All cases of AML, minimally differentiated (AMLmd) must have $<3\%$ positivity for MPO, Sudan

Black B (SBB), and naphthol ASD chloroacetate esterase by histochemical means. Many cases express lymphoid-associated markers such as CD2, CD7, and CD19 [15]. Of these lymphoid-associated markers, CD7 is the one found most commonly. Early hematopoietic markers (HLA-DR, CD34, and CD38) are usually expressed. There is usually no expression of later myeloid antigens such as CD15, CD14, or CD11b. Although there may be residual normal marrow including maturing neutrophil precursors, blasts are plentiful in most cases, with blast percentages on the order of 90% or more commonly seen. By light microscopy, there is no evidence of myeloid differentiation. Auer rods are not demonstrated. Electron microscopy can be used to demonstrate the myeloid nature of AMLmd but this method is not used commonly in the diagnosis of acute leukemia.

Acute myeloid leukemia without maturation

There is a logical progression as the WHO classification, like the FAB classification, moves toward leukemias with more evidence of differentiation. Acute myeloid leukemia without maturation [1] (FAB M1) [2,14] continues with a high percentage of blasts (typically more than 90% of all non-erythroid cells in the bone marrow) but shows evidence of myeloid differentiation by the presence of Auer rods or reactivity with MPO or SBB ($>3\%$) by histochemistry. Flow cytometry is still the best method for phenotyping this category of leukemia, but the markers will not distinguish AML without maturation (AML−m) from AML with maturation (AML + m) or AML, minimally differentiated. The blasts will be positive for two or more myeloid markers such as CD33, CD13, or CD117 and may express MPO by flow cytometry. Typically CD34 is expressed. Like AMLmd, later myeloid markers and those of monocytic differentiation are not seen in most cases. Lymphoid-associated antigens may be present but not lymphoid-restricted antigens.

Acute myeloid leukemia with maturation

Continuing the trend toward increasing differentiation, AML with maturation [1] (FAB M2) [2,14] has fewer blasts and must have at least 10% neutrophil precursors in the bone marrow showing maturation from the promyelocyte stage to segmented neutrophils. Some cases have increased numbers of eosinophil precursors, basophils, or mast cells. These cells are morphologically normal. Auer rods are relatively commonly seen in AML + m. This form of leukemia may not have more than the minimum percentage of blasts necessary to establish the diagnosis of acute leukemia (20%). It is the presence of more than 10% maturing forms in the neutrophil series that distinguishes this category from AML−m and the lack of a major monocytic component ($<20\%$) that separates AML + m from acute myelomonocytic leukemia (AMML). Flow cytometry will not always allow this separation with the antibody panels usually used. Myeloblasts in AML with maturation are positive for myeloid-associated antigens such as CD33, CD13, MPO, and CD15 [1,12]. In some cases using markers typically found on mature neutrophils such as the isoforms of CD66 can aid in the demonstration of

maturation. Markers of early hematopoietic cells like CD34, HLA-DR, and CD117 may be positive. Lymphoid-associated antigens may be present.

Acute myelomonocytic leukemia

This leukemia is composed of precursors of monocyte lineage and neutrophil lineage. To separate acute myelomonocytic leukemia [1] (FAB M4) [2,14] from chronic myelomonocytic leukemia [3] it is necessary to have at least 20% blasts present in the bone marrow or peripheral blood. Myeloblasts and more mature neutrophil precursors make up at least 20% of the cells in the bone marrow. Auer rods may be present. Monocytes, promonocytes, and monoblasts also total at least 20% of the cells in the bone marrow. The peripheral blood may have a significant monocytosis (5×10^9/L). The WHO classification [1] calls for at least 3% of the blasts to be positive for MPO by histochemical staining, and notes that the monocytic component should be non-specific esterase (NSE)-positive, although this is not an absolute requirement. The myeloid component in AMML will mark with the expected myeloid-associated antigens by flow cytometry, such as CD33, CD13, and CD117, and may mark with MPO. Flow cytometry will also demonstrate the monocytic elements by staining with antibodies such as CD14, CD64, and lysozyme, which are more specific for monocytic differentiation, and CD4, CD11b, CD11c, and CD36, which are less specific for monocytic differentiation. The myeloblasts are more likely to mark with CD34 than the monoblasts.

Acute monoblastic leukemia and acute monocytic leukemia

Acute monoblastic leukemia [1] (FAB M5a) [2,14] and acute monocytic leukemia [1] (FAB M5b) [2,14] must have at least 80% of the bone marrow cells to be of monocytic lineage. Neutrophil precursors comprise less than 20% of the bone marrow cells. Acute monoblastic leukemia (AMbL) is composed of monoblasts (more than 80% of the monocytic precursors) [1], whereas acute monocytic leukemia (AMoL) has fewer monoblasts and a greater percentage of promonocytes. It is uncommon to identify Auer rods in either AMbL or AMoL. Most cases of both varieties of acute leukemia will react strongly with the NSE stain. By flow cytometry, these leukemias may express those myeloid antigens shared between monocytic forms and granulocytic forms, such as CD33, CD13, and CD117. Most cases will be positive for at least some of the antigens associated with monocytic lineage: CD64, CD14, CD4, CD11b, CD11c, CD36, CD68, and lysozyme. Many cases of AMbL and AMoL are CD34-negative. Antibody against MPO will react with some cases of AMoL but fewer cases of AMbL [11].

Acute erythroid leukemia

Although the FAB only recognized one type of erythroleukemia (FAB M6) [2,14], the WHO classification recognizes a form that is entirely erythroid, called pure erythroid leukemia [1] (PEL), and the mixed myeloid and erythroid form recognized by the FAB that the WHO also calls erythroleukemia [1] (EL). The

erythroid component of each form reacts with antibody against hemoglobin A and glycophorin A by flow cytometry. In some cases of PEL, the most primitive erythroblasts may not mark with antibody against glycophorin A or hemoglobin A [1]. Other markers that have been used in such cases include carbonic anhydrase, CD36, and the Gero antibody against the Gerbich blood group [1]. Megakaryocytes and monocytes also mark with CD36; however it is measured, MPO is negative in the erythroblasts.

With erythroleukemia, more than 50% of the nucleated cells in the bone marrow are of erythroid origin. Of the non-erythroid cells, at least 20% must be myeloblasts with at least 3% positive for MPO or SBB. These myeloblasts mark by flow the same as myeloblasts in AML with or without maturation, that is, they are positive with at least some of these markers: CD33, CD13, CD117, and MPO [1,13]. Although the myeloblasts commonly mark with HLA-DR and CD34, the erythroblasts do not.

Pure erythroid leukemia [1] is a malignant proliferation of erythroblasts, frequently numbering more than 80% of the bone marrow cells. These cases do not have the myeloblastic proliferation of FAB erythroleukemia [2,14] or the WHO EL [1]. In those cases that do not mark by flow with antibody to glycophorin A or hemoglobin A, immunohistochemistry against hemoglobin A may be positive on the bone marrow biopsy [13]. Before the advent of the WHO classification, no matter how malignant the process looked or behaved, it was not considered an acute erythroid leukemia unless there were enough myeloblasts to meet the criteria for acute leukemia [2,14]. Without 30% blasts present in the non-erythroid bone marrow cells, the process was considered a myelodysplastic syndrome. With the revision of 1985 [14] the FAB did allow the blast count to be 30% of the non-erythroid bone marrow cells.

Acute megakaryoblastic leukemia

Like AML, minimally differentiated, acute megakaryoblastic leukemia [1] (FAB M7) [14] was not a part of the original FAB classification of acute leukemias. The ability to recognize megakaryocytic lineage has improved from the absolute need for electron microscopy to perform the platelet peroxidase stain to the readily available use of CD41 and CD61 by flow cytometry. At least half of the blasts must be of megakaryocytic lineage for the diagnosis of acute megakaryoblastic leukemia (AMgL) [1]. Histochemical stains for MPO and SBB are negative in megakaryoblasts. Like most forms of AML, CD33 and CD13 may be positive by flow cytometry. Although TdT is not found in AMgL [9], CD7 may be expressed. Most cases of AMgL are negative for CD34 and HLA-DR, and many are negative for CD45 also [1]. If one always chooses the cells on which to gate from a plot of CD45 versus side scatter [8], the blasts may not be readily recognized. It is always helpful to look at a well-stained smear, whether peripheral blood or bone marrow aspirate, and to look at a plot of forward scatter versus side scatter in addition to the widely accepted plot of CD45 versus side scatter. Most commonly CD41 and CD61 are examined for surface expression but cytoplasmic staining is more specific and this eliminates the

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quandary concerning whether the staining is real or represents platelet membranes adherent to the blasts.

The WHO recognizes a variant of AMgL seen in children with trisomy 21 or Down syndrome. These patients may present when neonates with a transient myeloproliferative disorder (TMD) that spontaneously resolves, or with AML around one year of age [7]. This is not a common condition but most cases mark by flow cytometry in a pattern similar to that of AMgL in some series [1]. Cases marking with erythroid markers have also been reported. A study by Karandikar et al [7] found the blasts to express CD33, CD38, and CD45 invariably, and most cases expressed CD34, CD7, and CD56. In their hands, all of the TMD and most of the cases of AML expressed CD36 and most cases expressed CD41 and CD61. These results do contrast with those discussed by the WHO in their new classification [1].

Acute myeloid leukemia with multilineage dysplasia

In addition to fulfilling the criteria for any class of acute leukemia, acute myeloid leukemia with multilineage dysplasia (AML-MD) [1] must have more than half the cells in at least two myeloid lines (granulocytic, megakaryocytic, and erythroid) displaying dysplasia. These patients typically present with marked pancytopenia. AML-MD may follow a myelodysplastic syndrome or a myelodysplastic/myeloproliferative syndrome, whereas some cases present de novo. It is no surprise with the amount of dysplasia involving two or all three of the myeloid cells lines that the blasts in AML-MD express a wide range of myeloid-associated antigens by flow cytometry, sometimes in unique, aberrant patterns or combinations. Most cases will mark with CD33 and CD13. Many cases express CD34. CD7 is commonly expressed. Aberrant expression of CD56 is not uncommon. There is nothing diagnostic about the pattern of staining with flow cytometry, but at the time unusual patterns of myeloid-associated antigens are found positive, significant amounts of dysplasia are commonly found by light microscopy.

The WHO [1] calls for the modifier "therapy-related" to be added to any diagnosis of myelodysplastic syndrome or acute myeloid leukemia wherein there is a history of treatment with radiation, alkalating agents, or topoisomerases. This designation does not equate to AML-MD. Typically the therapy-related leukemias and myelodysplastic syndromes do not differ in appearance from the acute leukemias not otherwise categorized, or from those with specific genetic abnormalities. First the acute leukemia or MDS is classified by the usual criteria using light microscopy, flow cytometry, and cytogenetics, then the modifier "therapy-related" is added.

Acute panmyelosis with myelofibrosis

This new entity adopts the old term panmyelosis to indicate the malignant proliferation of all three myeloid cell lines (granulocytes, megakaryocytes, and erythroid cells). There are significant amounts of fibrosis that may hamper efforts at flow cytometry. A fresh sample of bone marrow biopsy can be submitted and

in some cases enough cells can be freed to obtain reasonable results by flow cytometry. In those cases in which not enough cells are available for flow cytometry, immunophenotyping must be performed on the bone marrow biopsy by immunohistochemistry. Particularly on the biopsy, abnormal megakaryocytes are often prominent. With an expansion of all three cells lines, most of the myeloid-associated antigens may be expressed. There is frequently expression of CD33, CD13, CD117, and MPO. CD34 may be positive. A portion of the blasts will usually mark with megakaryocyte-associated or erythroid-associated antigens [1].

Myeloid sarcoma

Myeloid sarcoma represents a tumorous growth of immature myeloid precursors. This process has been reported presenting in most locations. There may be a preceding or concurrent hematologic condition [1], most commonly AML or chronic myeloid leukemia (CML) but other myeloproliferative disorders or even a myelodysplastic syndrome may lead to this manifestation. This group of disorders can involve myeloblasts and neutrophil precursors, monoblasts, or be a trilineage myeloid tumor of erythroid precursors, megakaryocytic precursors, and granulocytic precursors. The flow cytometric pattern will depend on the cell lines involved with most expressing myeloid-associated antigens. A non-specific but sensitive marker for myeloid sarcoma is positivity for CD43 in the absence of CD3. The WHO divides what they refer to as granulocytic sarcoma into three categories [1] based on cell composition. The blastic granulocytic sarcoma shows almost exclusively myeloblasts. With the immature form of granulocytic sarcoma there is a mixture of promyelocytes and myeloblasts. The form composed of maturing neutrophils and promyelocytes is called differentiated granulocytic sarcoma.

Acute basophilic leukemia

Basophilic precursors compose this rare form of acute leukemia. Some cases of acute basophilic leukemia [1,17] (ABL) result from a blast crisis of CML. Those cases should show the Philadelphia chromosome or the BCR/ABL fusion gene. By flow cytometry the blasts mark with CD33 and CD13 and are frequently positive for CD34 and HLA-DR [17]. Many cases of ABL will express CD9 and some will express CD7 or CD10 [23]. This pattern of antigens detected by flow cytometry is not specific for ABL. The morphology is helpful and metachromatic staining with toluidine blue is the best method of proving basophilic origin short of electron microscopy.

Acute myeloid leukemia with recurrent cytogenetic abnormalities

With well-controlled prospective clinical trials treating AML, it has become apparent that some specific cytogenetic changes associated with de novo development of AML have a more favorable prognosis. One form, acute promyelocytic leukemia is treated with an agent (all trans-retinoic acid) promot-

ing maturation of the leukemic cells. Other forms have a high complete response rate and better survival figures. Flow cytometry can aid in the phenotyping of these forms of AML but in and of itself, flow cytometry is not the primary diagnostic tool. Even the percentage of blasts is not as important as the presence of the specific cytogenetic abnormality [1].

Acute promyelocytic leukemia with t(15;17)(q22;q12), (PML/RAR) and its variants

In acute promyelocytic leukemia [1] (FAB M3) [2,14] abnormal promyelocytes are the proliferating cells with few myeloblasts typically identified. One of the hallmarks of this category of acute leukemia is abundant Auer rods, frequently in bundles. There are two forms of acute promyelocytic leukemia (APL), the usual hypergranular form and the less common microgranular form in which the primary granules may not be recognized by routine light microscopy. Both show the same characteristic bilobed or kidney shaped nuclei. APL marks with myeloid-associated antigens by flow cytometry. Typically acute promyelocytic leukemia is negative against antibodies to CD34 and HLA-DR [1,12]. The promyelocytes commonly mark with CD9 and CD2. CD15 may be present but more often it is weak or negative [1]. It has been reported that CD15 is never coexpressed with CD34 in APL [1]. Although there are variants for the translocation, all involving chromosome 17, the classic form is a t(15;17)(q22;q12). The diagnosis of APL is based on the morphology coupled with traditional cytogenetics. Flow cytometry plays an ancillary role but is not diagnostic.

Acute myeloid leukemia with t(8;21)(q22;q22), (AML1/ETO)

The most common morphologic manifestation of AML with t(8;21)(q22;q22) [1] is AML with maturation. Although eosinophils may be increased in this form of acute leukemia, they do not show abnormal morphology. AML with t(8;21)(q22;q22) has the phenotype that goes with its morphologic manifestation. A high percentage of these cases express CD19 on at least a portion of the blasts. CD56 is present on a significant percentage of cases and may confer a worse prognosis. With the presence of the t(8;21)(q22;q22), this process is considered an acute leukemia even when the myeloblast percentage is less than 20% [1].

Acute myeloid leukemia with inv(16)(p13;q22) or t(16;16)(p13;q22), (CBF/MYH11)

Abnormal eosinophils with larger granules that often stain a bluish-purple color are the most striking morphologic feature in this category of leukemia. AML with inv(16)(p13;q22) or t(16;16)(p13;q22) [1] has the morphology and flow cytometric immunophenotype of AMML. It is usually designated AMML-Eo [4]. By flow cytometry most of the blasts mark with CD33 and CD13. The myeloblasts mark with MPO whereas the monoblasts mark with some or all of CD4, CD11b, CD11c, CD14, CD36, CD64, CD68, and lysozyme. CD2 is expressed more often than CD7 on the blasts.

Acute myeloid leukemia with 11q23 (MLL) abnormalities

In contrast to the other forms of acute myeloid leukemia with recurrent cytogenetic abnormalities discussed before, the prognosis is not as favorable in the presence of 11q23 translocations or deletions [1]. The morphologic manifestation is usually that of monocytic differentiation, with AMoL and AMML the most common categories seen. The immunophenotype is that of the morphologic manifestation whether AMML or AMoL. There is an association with prior topoisomerase therapy and 11q23 abnormalities [1].

Acute leukemia of ambiguous lineage

This grouping of leukemias relies heavily on the proof of lineage. Flow cytometry is of prime importance in establishing the diagnosis of bilineal or biphenotypic acute leukemia. Care must be taken in making the diagnosis of any leukemia in which the lineage is not clear [4,5]. As can be seen in the acute myeloid leukemias discussed earlier, lymphoid-associated antigens are by no means infrequently expressed in AML.

Undifferentiated acute leukemia

When no lineage-specific antigens are detected in a leukemia with at least 20% blasts, then the diagnosis of undifferentiated acute leukemia (UAL) [1] may be entertained. To fulfill the WHO criteria, there should be no expression of MPO, cyCD3, cyCD22, or cyCD79a. By flow cytometry most cases will only be positive with one lineage-associated antigen such as CD33 or CD13. Many cases of UAL will be positive with CD34, HLA-DR, and CD38. Some cases will have TdT and CD7 positivity.

Bilineal acute leukemia

To categorize an acute leukemia as bilineal [1], it is necessary to demonstrate blasts from two distinct lineages. Flow cytometry is the easiest method to achieve this end. The blasts may be of myeloid origin and either B-lymphocytic or T-lymphocytic origin, or may be of T-lymphocytic origin and B-lymphocytic origin and not be myeloid at all. The best proof of myeloid origin is positivity for MPO antigen or reactivity in at least 3% of blasts by the histochemical stain for MPO. To prove T-lymphocytic origin, cytoplasmic CD3 needs to be identified. For B-lymphocytic origin, flow cytometry should demonstrate cytoplasmic CD79a or cytoplasmic CD22.

Biphenotypic acute leukemia

Flow cytometry using at least two-color analysis is required to make a diagnosis of biphenotypic acute leukemia [1]. The blasts are positive for markers specific for two distinct lineages or, less commonly, all three lineages. Flow cytometry will demonstrate MPO for myeloid lineage and either cyCD3 for T-lymphocytic lineage or cyCD79a or cyCD22 for B-lymphocytic lineage or the blasts may express cyCD3 and cyCD79a or CCD22 to be B- and T-lymphocytic

in nature. This class of leukemia is rare, and finding MPO, cyCD3, and cyCD79a/cyCD22 on the same blast cells is extremely uncommon but does happen. It is much more common to find acute lymphoblastic leukemia with one or two myeloid-associated antigens present or acute myeloid leukemia with one or two lymphoid-associated antigens present. Surface staining by flow cytometry for CD3 does not have the significance of cyCD3.

Myelodysplastic syndromes

This category of disorders is defined by the presence of dysplasia and ineffective hematopoiesis [3]. As demonstrated frequently by cytogenetics, these are clonal processes that may lead to AML, if the progressive failure of the bone marrow does not result in death first. The dysplasia may involve any myeloid cell line or all of them. Flow cytometry can provide useful information but is not diagnostic for any class of myelodysplastic syndrome (MDS). Chang and Cleveland [18] have demonstrated decreased numbers of segmented neutrophils expressing CD10 in MDS when compared with normal bone marrow aspirates. There are reports [19] of altered levels of leukocyte alkaline phosphatase in cases of myelodysplastic syndromes. Flow cytometry can detect this change and is much less time consuming and more reproducible than the manual method of detection.

Refractory anemia

As with the acute myeloid leukemias not otherwise categorized, there is a progression of the findings in the diagnosis of MDS. Refractory anemia (RA) [1,3] has anemia as its main peripheral manifestation. The dysplasia frequently is seen only in the erythroid series and may be subtle. There are $<1\%$ blasts in the peripheral blood and $<5\%$ blasts in the bone marrow by light microscopy or flow cytometry. If ringed sideroblasts are present in the bone marrow, they must number $<15\%$ of the nucleated erythroid cells. The blasts present will mark with myeloid-associated markers.

Refractory anemia with ringed sideroblasts

The only difference between RA and refractory anemia with ringed sideroblasts (RARS) [1,3] is the presence in the bone marrow aspirate of ringed sideroblasts in 15% or more of the nucleated erythroid cells. Flow cytometry will confirm the percentage of blasts determined by manual differential on peripheral blood or bone marrow but adds little to the diagnosis beyond the ability to demonstrate aberrant phenotypes of the blasts present.

Refractory cytopenia with multilineage dysplasia

Refractory cytopenia with multilineage dysplasia (RCMD) [1] continues with the same blast requirements, that is, $<1\%$ blasts in the peripheral blood and $<5\%$ blasts in the bone marrow. Patients present with cytopenia in at least two cell lines and many show pancytopenia. With the presence of dysplasia in at least 10% of the cells in at least two of the myeloid cell lines (erythroid, megakaryocytic, and granulocytic), the absence of Auer rods becomes more germane. Also, there must be $<1 \times 10^9$/L monocytes in the peripheral blood. As with RA, if there are ringed sideroblasts in the bone marrow, the ringed sideroblasts in RCMD will be less than 15% of the nucleated erythroid cells. Beyond confirming the percentage of blasts and detecting aberrant phenotypes present on blasts, flow cytometry plays no real diagnostic role in RCMD.

Refractory cytopenia with multilineage dysplasia with ringed sideroblasts

This category of MDS is identical to RCMD with the addition of 15% or more ringed sideroblasts in the bone marrow aspirate iron stain [1]. As with the other categories of MDS, the WHO classification states that flow cytometry and other methods of immunophenotyping are not relevant to the diagnosis. Morphology is the key to the diagnosis in all of the categories of MDS but flow cytometry can confirm blast percentages and identify aberrant populations of cells present in the samples analyzed.

Refractory anemia with excess blasts

The WHO divides what the FAB labeled as refractory anemia with excess blasts (RAEB) [1,3] into two subtypes based on percentage of blasts in the peripheral blood and the bone marrow. RAEB-1 [1] has $<5\%$ blasts in the peripheral blood and 5–9% blasts in the bone marrow. RAEB-2 [1] has 5–19% blasts in the peripheral blood and 10–19% blasts in the bone marrow. Both types of RAEB typically present with pancytopenia. Auer rods will move a case otherwise fitting the criteria for RAEB-1 into the category of RAEB-2. Both types of RAEB have $<1 \times 10^9$/L monocytes in the peripheral blood. With increasing percentages of blasts, flow cytometry can be more helpful in confirming the number of blasts. There is also a greater chance of detecting populations of cells with aberrant phenotypes. The blasts usually mark with myeloid-associated markers such as CD33, CD13, and CD117.

Myelodysplastic syndrome, unclassifiable

When the peripheral blood and bone marrow show significant dysplasia in one or more myeloid cell lines, the diagnosis of a myelodysplastic syndrome seems in order. Some cases do not fit well into one of the previously mentioned categories of MDS. To be categorized as MDS, unclassifiable, there must be $<1\%$ blasts in the peripheral blood and $<5\%$ blasts in the bone marrow. To separate this disorder from chronic myelomonocytic leukemia (CMML), there must be $<1 \times 10^9$/L monocytes in the peripheral blood. Auer rods exclude any case from the diagnosis of MDS, unclassifiable. Immunophenotyping may aid in confirming the percentage of blasts and will reveal cells with aberrant marker patterns when present.

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Myelodysplastic syndrome associated with isolated del(5q) chromosome abnormality

Although many cases of MDS have translocations or deletions with no effect on the categorization of the MDS, the presence of an isolated del(5q) [1] has been singled out by the WHO for its good prognosis relative to other kinds of MDS. Anemia is frequently the only cytopenia and, in contrast to most myelodysplastic syndromes, those with del(5q) have thrombocytosis more often than thrombocytopenia. The bone marrow blast count remains <5% but the peripheral blast percentage is also <5% instead of the <1% common to several of the MDS. Auer rods should not be present. The flow cytometry frequently reveals the same myeloid-associated antigens seen in other MDS. Aberrant phenotypes may be a little less common with this category of MDS.

Myelodysplastic/myeloproliferative diseases

There are several disorders that have been poorly understood or that do not fit any previously defined category of disease. Philadelphia chromosome-negative chronic myeloid leukemia is a prime example of this new grouping of diseases. These processes share features from myelodysplastic syndromes and myeloproliferative diseases. Most often the peripheral blood shows a leukocytosis, thrombocytosis, or less commonly, an erythrocytosis. There may be coexisting cytopenias also. Dysplasia is a feature of this class of diseases, although it may not be prominent. As is typical of myeloproliferative diseases and myelodysplastic syndromes, the bone marrow tends to be hypercellular. Flow cytometry will clarify the phenotype of the cells proliferating in myelodysplastic/myeloproliferative diseases (MDS/MPD) [1] and the blasts present. All of these diseases have less than 20% blasts by definition [1]. Cells with aberrant phenotypes may be detected in any of these disorders. There is no specific phenotype that is diagnostic of this class of diseases.

Atypical chronic myeloid leukemia

Atypical chronic myeloid leukemia (aCML) [1] has been recognized by various names for many years. The most descriptive term used in the past is Philadelphia chromosome-negative CML. As this term indicates, the presence of the Philadelphia chromosome (or the BCR/ABL fusion gene) must be absent to entertain the diagnosis of aCML. Either name for this disease reflects the neutrophilic leukocytosis with a left-shift. Usually there are promyelocytes and, not infrequently, myeloblasts present in the peripheral blood. The percentage of blasts allowed in the peripheral blood should be <5% and there must be <20% blasts in the bone marrow. At least 10% of the peripheral blood leukocytes must be in the neutrophil series. The neutrophil precursors show dysplasia in the bone marrow and the peripheral blood and usually it is conspicuous. Basophilia is not a feature of this process. Although there is not a specific immunophenotype for the disease, flow cytometry marks the blasts with myeloid-associated antigens and may reveal an aberrant phenotype. The patients with aCML have a more aggressive course than Philadelphia chromosome-positive CML patients.

Chronic myelomonocytic leukemia

The FAB classified what is called chronic myelomonocytic leukemia (CMML) [1,3] as a myelodysplastic syndrome. The features necessary to make the diagnosis of CMML include the absence of the Philadelphia chromosome or the BCR/ABL fusion gene. There is a monocytosis of at least 1×10^9/L. As is common to all of these diseases, there must be <20% blasts in the peripheral blood and bone marrow. This blast percentage includes monoblasts, promonocytes, and myeloblasts. Dysplasia must be present in at least one myeloid cell line. When all the other criteria for CMML are met but dysplasia is not prominent, the diagnosis of CMML can be made in the face of additional findings [1]. If a cytogenetic abnormality is demonstrated in the proliferative cells or if the monocytosis present has no identifiable cause and persists for longer than three months, then the diagnosis of CMML may be rendered.

Just as in RAEB, the WHO divides CMML into two subgroups [1] based on the percentage of blasts in the peripheral blood and bone marrow. Analogous to the subgroups of RAEB, CMML-1 has <5% blasts in the peripheral blood and <10% but usually >5% blasts in the bone marrow. CMML-2 has 5–19% blasts in the peripheral blood and 10–19% blasts in the bone marrow. It must be emphasized that promonocytes and monoblasts are added to myeloblasts in arriving at these percentages. CMML-2 is the appropriate diagnosis in the presence of Auer rods as long as the blast percentage is <20% in either the bone marrow or the peripheral blood. An additional subtype of CMML is CMML with eosinophilia [1]. It is unclear how significant this designation is to the patient beyond the diagnosis of CMML. The criteria for CMML must be met with the addition of an eosinophilia of 1.5×10^9/L for the diagnosis of CMML with eosinophilia, whether CMML-1 or CMML-2.

Flow cytometry will reveal expression of myeloid-associated markers (CD33 and CD13) in most cases. There is evidence of monocytic differentiation by flow cytometry. Not all monocyte-associated antigens will be positive in all cases of CMML but most will be positive for some of these markers, CD64, CD14, CD68, or lysozyme. The percentage of blasts is not as easily assessed by flow with the need to include promonocytes. Some cases will express CD5 and CD2 [1]. CD34 may be present on the blasts in both subtypes of CMML.

Juvenile myelomonocytic leukemia

As with CMML, there is an increase in peripheral blood monocytes to a level of at least 1×10^9/L. The blasts again include promonocytes and monoblasts in addition to the myeloblasts. For all of these diseases there must be <20% blasts in the bone marrow and in the peripheral blood. If the Philadelphia chromosome

or the BCR/ABL fusion gene is present, then the diagnosis is CML. Beyond these criteria two of the following additional criteria must be met for the diagnosis of juvenile myelomonocytic leukemia (JMML) [1]. The critera include a cytogenetic abnormality in the involved cells, or a left-shift in the neutrophilic series, shifted at least to the myelocyte stage, or a leukocytosis of at least 10×10^9/L. Other criteria are increased sensitivity in vitro to granulocyte-monocyte colony-stimulating factor involving myeloid progenitor cells, or a higher level of hemoglobin F for the patient's age [1]. This is a disease of children, with most cases occurring before age 14. Flow cytometry does not reveal a specific phenotype but can demonstrate cells with aberrant antigen expression. With promonocytes and monoblasts counted in the blast percentage, flow cytometry is not as helpful confirming the number of blasts.

Myelodysplastic/myeloproliferative disease, unclassifiable

Myelodysplastic/myeloproliferative disease, unclassifiable (MDS/MPD-U) [1] displays features of the myelodysplastic syndromes and of chronic myeloproliferative diseases (CMPD) but does not fulfill the exact criteria for any subtype of either class of disease. These cases must not fulfill the criteria for acute leukemia and must have no history of any preceding MDS or CMPD. Exposure to agents that could explain the proliferative aspects or the dysplasia must be excluded. There are specific cytogenetic deletions and translocations that must not be present. These include del(5q), the Philadelphia chromosome or the BCR/ABL fusion gene, inv(3)(q21;q26), and t(3;3)(q21;q26) [1]. Flow cytometry can aid in confirming the percentage of blasts and may demonstrate maturation of the involved cell lines. When cells are present with an aberrant phenotype, flow cytometry can detect these. There is no specific pattern of antigens associated with MDS/MPD-U.

Prognosis and flow cytometry

Various areas are actively being explored to define the prognosis and efficacy of therapeutic regimens for acute leukemias and myelodysplastic syndromes. The Southwest Oncology Group (SWOG) [20] has examined the role of multidrug resistance glycoprotein, multidrug resistance associated protein, and lung resistance protein in patients with AML, focusing on younger patients who typically can withstand the regimens better than older individuals can. This study used multiparameter flow cytometry to quantify these proteins before treatment was initiated. In the initial SWOG study [20], there was no correlation between the level of any of these three proteins and either overall survival or disease-free survival. A study from the University of Essen Medical School [21] in Germany showed similar results looking at multidrug resistance-related protein, heat-shock protein 27, bcl-2, and mutant p53. None of these proteins was an independent prognostic indicator. There was a significant correlation between response to therapy and the

detection of two or more of these proteins. Only the bcl-2 and multidrug resistance-related protein were detected by flow cytometry [21]. A brief report from Russia [22] states that detection of CD95 (Fas/APO-1) by flow cytometry in MDS confers a more favorable prognosis whereas those patients who were CD95-negative were at greater risk for progression to AML. The CD95 cases of MDS also tended to have a shorter survival whether or not they transformed into AML. One Swedish study [23] looking at CD14 expression in patients with AML found a negative effect on prognosis when 20% of the nonerythroid cells expressed the antigen. There was a significant shortening of patient survival for patients with at least 10% CD14-positive cells in FAB subtypes M0, M1, M2, and M3.

Minimal residual disease

From the earliest therapies inducing a complete remission in any acute leukemia, one question asked was "Is the patient in remission?" By light microscopy, detection of potential residual disease is on the order of 10^{-2} [24]. With the advent of flow cytometry the level of detection has been lowered to 10^{-3}–10^{-5} cells [24]. Polymerase chain reaction gives similar to slightly better detection rates. Flow cytometry reveals its best results when the leukemic cells have a unique or aberrant phenotype allowing for easier separation from normal regenerating bone marrow. In a study by Venditti et al [25], a level of 3.5×10^{-4} or higher leukemic cells after consolidation therapy was associated with unfavorable prognostic indicators and has a shorter duration of overall survival and disease-free survival in patients with AML. Other studies have found similar results but the level of minimal residual disease that is acceptable for the patient's long-term survival is still open to question.

Alternative methods for flow cytometry

The scope of this article is too limited to do justice to newer technologies building on the tried and true flow cytometer. One device that deserves brief mention is CompuCyte's laser scanning cytometer® (CompuCyte Corp; Cambridge, MA). As the astute reader will have observed, many of the diagnoses discussed in this article rely heavily on the morphology of the cells involved in these malignant conditions. Flow cytometry has many good features but it does not allow examination of the cells that mark with a particular pattern of antibodies. The laser scanning cytometer allows for the use of dual-labeled antibodies, a true necessity for phenotyping leukemic cells. The advantage of the laser scanning cytometer is the ability to examine the cells marking with a particular antibody or pair of antibodies by way of an integrated microscope or on the computer screen. Among other uses, this would help in looking at small populations of cells with aberrant phenotypes and may aid in excluding adherence of platelet membranes to blasts when diagnosing AMgL.

Summary

Flow cytometry is the primary tool for phenotyping leukemias and related conditions. With the ever increasing numbers of antibodies commercially available, the ability to study and understand leukemias, myelodysplastic syndromes, and the myelodysplastic/myeloproliferative diseases is improving. Although the data generated by flow cytometry are not comprehensive enough to completely subtype leukemias or myelodysplastic syndromes into their myriad divisions, this wealth of information does provide phenotype, reproducible enumeration of blasts, certain prognostic information, and it can reveal the presence of cell populations with aberrant antigen profiles. An important drawback to flow cytometry as it is performed today is the inability to look at the cells that mark with the antibody panels used. As classifications of leukemia and related conditions evolve, flow cytometry continues to answer many of the questions asked and to provide critical information reliably and quickly.

References

[1] Jaffe ES, Harris NL, Stein H, Vardiman JW, editors. Pathology and genetics of tumours of haematopoietic and lymphoid tissues. World Health Organization classification of tumours. 3rd edition. Lyon, France: International Agency for Research on Cancer; 2001.

[2] Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DAG, Gralnick HR, et al. Proposals for the classification of the acute leukemias. Br J Haematol 1976;33:451–8.

[3] Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DAG, Gralnick HR, et al. Proposals for the classification of the myelodysplastic syndromes. Br J Haematol 1982;51:189–99.

[4] Kaleem Z, White G. Diagnostic criteria for minimally differentiated acute myeloid leukemia (AML-M0). Am J Clin Pathol 2001;115:876–84.

[5] Kotylo PK, Seo IS, Smith FO, Heerema NA, Fineberg NS, Miller K, et al. Flow cytometric immunophenotypic characterization of pediatric and adult minimally differentiated acute myeloid leukemia (AML-M0). Am J Clin Pathol 2000;113:193–200.

[6] Krasinskas AM, Wasik MA, Kamoun M, Schretzenmair R, Moore J, Salhany KE. The usefulness of CD64, other monocyte-associated antigens, and CD45 gating in the subclassification of acute myeloid leukemias with monocytic differentiation. Am J Clin Pathol 1998;110:797–805.

[7] Karandikar NJ, Aquino DB, M^{c}Kenna RW, Kroft SH. Transient myeloproliferative disorder and acute myeloid leukemia in Down syndrome. Am J Clin Pathol 2001;116:204–10.

[8] Lacombe F, Durrieu F, Briais A, Dumain P, Belloc F, Bascans E, et al. Flow cytometry CD45 gating for immunophenotyping of acute myeloid leukemia. Leukemia 1997;11:1878–86.

[9] Huh YO, Smith TL, Collins P, Bueso-Ramos C, Albitar M, Kantarjain HM, et al. Terminal deoxynucleotidyl transferase expression in acute myelogenous leukemia and myelodyplasia as determined by flow cytometry. Leuk Lymphoma 2000;37:319–31.

[10] Konikova E, Glasova M, Kusenda J, Babusikova O. Intracellular markers in acute myeloid leukemia diagnosis. Neoplasma 1998;45:282–91.

[11] Nakase K, Sartor M. Detection of myeloperoxidase by flow cytometry in acute leukemia. Cytometry 1998;34:198–202.

[12] Dunphy CH, Polski JM, Evans HL, Gardner LJ. Evaluation of bone marrow specimens with acute myelogenous leukemia for CD34, CD15, CD117, and myeloperoxidase: comparison of flow cytometry, enzyme cytochemical versus immunohistochemical techniques. Arch Pathol Lab Med 2001;125: 1063–9.

[13] Manaloor EJ, Neiman RS, Heilman DK, Albitar M, Casey T, Vattuone T, et al. Immunohistochemistry can be used to subtype acute myeloid leukemias in routinely processed bone marrow biopsy specimens. Am J Clin Pathol 2000;113:814–22.

[14] Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DAG, Gralnick HR, et al. Proposed revised criteria for the classification of acute myeloid leukemia. Ann Int Med 1985;103:620–5.

[15] Launder TM, Bray RA, Stempora L, Chenggis ML, Farhi DC. Lymphoid-associated antigen expression by acute myeloid leukemia. Am J Clin Pathol 1996;106:185–91.

[16] Kraguljac N, Marisavljevic D, Jankovic G, Radosevic N, Pantic M, Donfrid M, et al. Characterization of CD13 and CD33 surface antigen-negative acute myeloid leukemia. Am J Clin Pathol 2000;114:29–34.

[17] Peterson LC, Parkin JL, Arthur DC, Brunning RD. Acute basophilic leukemia: a clinical, morphologic, and cytogenetic study of eight cases. Am J Clin Pathol 1991;96:160–70.

[18] Chang CC, Cleveland RP. Decreased CD10 positive mature granulocytes in bone marrow from patients with myelodysplastic syndrome. Arch Pathol Lab Med 2000;124:1152–6.

[19] Okamoto T, Okada M, Yamada S, Fujimori Y, Takemoto Y, Masuhara K, et al. Flow-cytometric analysis of leukocyte alkaline phosphatase in myelodysplastic syndromes. Acta Haematol 1999; 102:89–93.

[20] Leith CP, Kopecky KJ, Chen IM, Eijdems L, Slovak ML, M^{c}Connell TS, et al. Frequency and clinical significance of the expression of the multidrug resistance protein MDR1/P-glycoprotein, MRP1, and LRP in acute myeloid leukemia: a Southwest Oncology Group study. Blood 1999; 94:1086–99.

[21] Kasimir-Bauer S, Ottinger H, Meusers P, Beelen DW, Bettinger G, Seeler S, et al. In acute myeloid leukemia, coexpression of at least two proteins, including P-glycoprotein, the multidrug resistance-related protein, bcl-2, mutant p53, and heat-shock protein 27, is predictive of the response to induction chemotherapy. Exp Hematol 1998;26:1111–7.

[22] Polosukhina ER, Kuznetsov SV, Logcheva NP, Zaborina TN, Tenuta MR, Shirin AD, et al. An evaluation of the prognostic significance of antigen CD95 (Fas/APO-1) expression on the cells of patients with a myelodysplastic syndrome, acute myeloid leukemia and chronic myeloleukemia. Ter Arkh 1998;70:21–5.

[23] Fergdal M, Astrom M, Tidefelt U, Karlsson MG. Differences in CD14 and alpha-naphthyl acetate esterase positivity and relation to prognosis in AML. Leuk Res 1998;22:25–30.

[24] Faderl S, Kurzrock R, Estrov Z. Minimal residual disease in hematologic disorders. Arch Pathol Lab Med 1999;123:1030–4.

[25] Venditti A, Buccisano F, Del Poeta G, Maurillo L, Tamburini A, Cox C, et al. Level of minimal residual disease after consolidation therapy predicts outcome in acute myeloid leukemia. Blood 2000;96:3948–52.

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[6] Krasinskas AM, Wasik MA, Kamoun M, Schretzenmair R, Moore J, Salhany KE. The usefulness of CD64, other monocyte-associated antigens, and CD45 gating in the subclassification of acute myeloid leukemias with monocytic differentiation. Am J Clin Pathol 1998;110:797–805.

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[11] Nakase K, Sartor M. Detection of myeloperoxidase by flow cytometry in acute leukemia. Cytometry 1998;34:198–202.

[12] Dunphy CH, Polski JM, Evans HL, Gardner LJ. Evaluation of bone marrow specimens with acute myelogenous leukemia for CD34, CD15, CD117, and myeloperoxidase: comparison of flow cytometry, enzyme cytochemical versus immunohistochemical techniques. Arch Pathol Lab Med 2001;125: 1063–9.

[13] Manaloor EJ, Neiman RS, Heilman DK, Albitar M, Casey T, Vattuone T, et al. Immunohistochemistry can be used to subtype acute myeloid leukemias in routinely processed bone marrow biopsy specimens. Am J Clin Pathol 2000;113:814–22.

[14] Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DAG, Gralnick HR, et al. Proposed revised criteria for the classification of acute myeloid leukemia. Ann Int Med 1985;103:620–5.

[15] Launder TM, Bray RA, Stempora L, Chenggis ML, Farhi DC. Lymphoid-associated antigen expression by acute myeloid leukemia. Am J Clin Pathol 1996;106:185–91.

[16] Kraguljac N, Marisavljevic D, Jankovic G, Radosevic N, Pantic M, Donfrid M, et al. Characterization of CD13 and CD33 surface antigen-negative acute myeloid leukemia. Am J Clin Pathol 2000;114:29–34.

[17] Peterson LC, Parkin JL, Arthur DC, Brunning RD. Acute basophilic leukemia: a clinical, morphologic, and cytogenetic study of eight cases. Am J Clin Pathol 1991;96:160–70.

[18] Chang CC, Cleveland RP. Decreased CD10 positive mature granulocytes in bone marrow from patients with myelodysplastic syndrome. Arch Pathol Lab Med 2000;124:1152–6.

[19] Okamoto T, Okada M, Yamada S, Fujimori Y, Takemoto Y, Masuhara K, et al. Flow-cytometric analysis of leukocyte alkaline phosphatase in myelodysplastic syndromes. Acta Haematol 1999; 102:89–93.

[20] Leith CP, Kopecky KJ, Chen IM, Eijdems L, Slovak ML, M^{c}Connell TS, et al. Frequency and clinical significance of the expression of the multidrug resistance protein MDR1/P-glycoprotein, MRP1, and LRP in acute myeloid leukemia: a Southwest Oncology Group study. Blood 1999; 94:1086–99.

[21] Kasimir-Bauer S, Ottinger H, Meusers P, Beelen DW, Bettinger G, Seeler S, et al. In acute myeloid leukemia, coexpression of at least two proteins, including P-glycoprotein, the multidrug resistance-related protein, bcl-2, mutant p53, and heat-shock protein 27, is predictive of the response to induction chemotherapy. Exp Hematol 1998;26:1111–7.

[22] Polosukhina ER, Kuznetsov SV, Logcheva NP, Zaborina TN, Tenuta MR, Shirin AD, et al. An evaluation of the prognostic significance of antigen CD95 (Fas/APO-1) expression on the cells of patients with a myelodysplastic syndrome, acute myeloid leukemia and chronic myeloleukemia. Ter Arkh 1998;70:21–5.

[23] Fergdal M, Astrom M, Tidefelt U, Karlsson MG. Differences in CD14 and alpha-naphthyl acetate esterase positivity and relation to prognosis in AML. Leuk Res 1998;22:25–30.

[24] Faderl S, Kurzrock R, Estrov Z. Minimal residual disease in hematologic disorders. Arch Pathol Lab Med 1999;123:1030–4.

[25] Venditti A, Buccisano F, Del Poeta G, Maurillo L, Tamburini A, Cox C, et al. Level of minimal residual disease after consolidation therapy predicts outcome in acute myeloid leukemia. Blood 2000;96:3948–52.