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Flowcytometry in Acute Leukemia

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Abstract

Acute leukemia is a heterogeneous group of malignancies with varying clinical, morphologic, immonophenotypic, genetic and molecular characteristics. Despite the increasing importance of molecular and genetic features in the sub-classification of acute leukemias, morphologic and immunophenotypic analysis remains the main modality to diagnose acute leukemia for initial evaluation and providing a rapid assessment to direct specific molecular genetic tests. There are many immonophenotypic markers which are associated with bad prognosis in acute leukemia. Flowcytometric immunophenotyping also help in deciding therapy with monoclonal antibodies directed against leukemia surface antigens including CD19, CD20, CD22, and CD52 which are particularly beneficial where further intensification of chemotherapy is impossible, particularly when there is minimal overlapping toxicity. In addition to that, flowcytometry is the main stay of evaluating minimal residual disease, particularly in cases without any specific molecular signature.

Keywords: Flowcytometric immunophenotyping; Leukemia associated phenotype; Minimal residual disease

Introduction

Flowcytometry provides an insight into differentiation pathways, maturation stages and abnormal features of the cell populations which are clinically relevant for the diagnosis of hematological malignancies. The presence and absence of antigens on or in the cell populations are recognized by various Monoclonal Antibodies (MAb) which gives characteristic immunostaining defining the cell lineages thus helping in making the diagnosis of acute leukemias [1]. Acute leukemia is a heterogeneous group of malignancies with varying clinical, morphologic, immonophenotypic, genetic and molecular characteristics. Flowcytometric immunophenotyping is a rapid reliable method not only to diagnose but also to assess prognosis, decision making for targeted therapy and follow up (in minimal residual disease evaluation) in acute leukemia. In spite of the increasing importance of molecular and genetic study in sub-classification of acute leukemia, molecular and genetic methods are available only in specialized reference laboratories and require a high level of technical expertise. So the morphology and immunophenotyping remain the main modalities for diagnosis.

Diagnosis

Flowcytometric immunophenotyping is useful in diagnosing the lineage in acute leukemia by the expression of lineage specific CD markers. Common leucocyte antigen (CD45) is used to gate the blast population and expression of the other lineage specific markers are analyzed on the gated population. CD45 expression is also different in different type of blasts e.g. B lymphoblasts are CD45 negative to moderate positive, myeloid blats are CD45 moderate with higher side scatter due to granularity, abnormal promyelocytes shows tear drop pattern in Acute Promyelocytic Leukemia (APML) and T lymphoblasts are CD 45 expression moderate to same as normal lymphocyte population (Figure 1). Common antibodies used in flowcytometric immunophenotyping of acute leukemia are: stem cell/hematopoietic precursors (CD34, HLA-DR, terminal deoxynucleotidyl transferase/TdT), myeloid markers (cMPO, CD13, CD33, CD117, CD15 (Figure 2), monocytic markers (CD64, CD14, CD11b,CD11c, lysozyme) (Figure 2), erythroid (CD71, CD235a), megakaryocytic (CD41, CD61, CD36), B lymphoid markers (CD19, CD10, CD20, CD22, cCD79a) (Figure 3), T lymphoid markers (CD3, CD5, CD7, CD1a,CD2, CD4, CD8) (Figure 4) and natural killer (NK) cells (CD56). Mixed phenotypic acute leukemia or trilineage acute leukemia can only be diagnosed on flowcytometric immunophenotyping by presence of more than one lineage markers. In acute myeloid leukemia with myelodysplastic syndrome, along with the morphological findings, the dysgranulopoiesis is noted on immunophenotyping as alter maturation patterns.

Apart from the lineage identification, flowcytometry may predict few genetic aberrations, which

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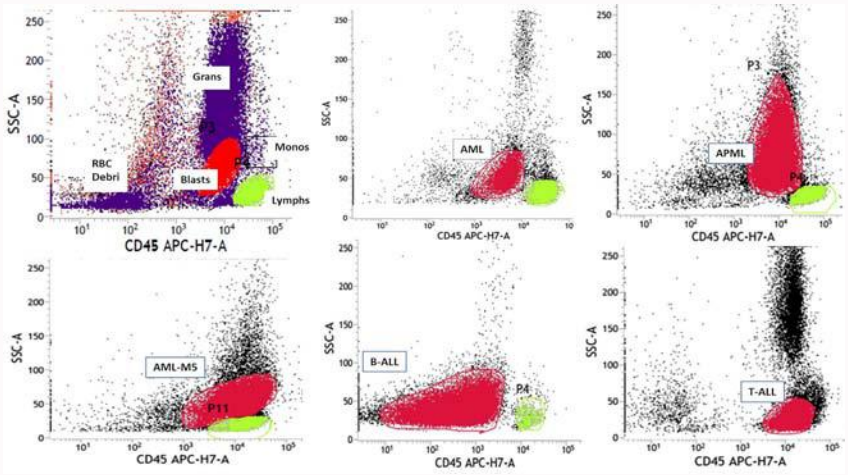

Figure 1: CD45 expression of different type of blasts: myeloblasts. CD45 moderate with higher side scatter, in APML tear drop pattern due to hypergrnulations, in AML M5 blasts in myelomonocytic region, B lymphoblasts with heterogenous dim CD45and T lymphoblasts with CD 45 expression same position as normal lymphocyte population.

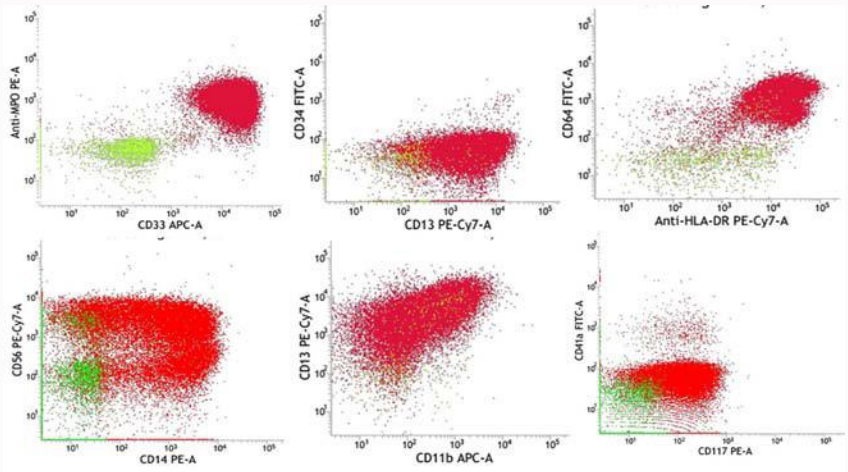

Figure 2: AML cases with positive myeloid and monocytic markers (blasts are represented by red colour).

can be confirmed on Fluorescence *in situ* Hybridization (FISH) and polymerase chain reaction (PCR). Acute myeloid leukemia with high side scatter (tear drop pattern) and lacking expression of CD34 and HLA-DR with bright CD33 and heterogeneous CD13, raises the suspicion of an APML to start immediate therapy with all-trans retinoic acid (ATRA), and can be confirm FISH for the t(15;17) and real time PCR for PML-RARA. They often express CD9 and lack CD18. Translocation (8,21) is associated with expression of B lymphoid markers as CD19 or CD79a. In Inversion 16 in addition to myeloid markers, they often express monocytic markers as CD14, CD64, CD4, CD11b, CD11c, CD36 and lysozyme. B- ALL with t(4,11) is usually associated with CD10 negative, frequently CD24 negative and positive for myeloid associated markers as CD15 and CD65. BCR-ABL positive B-ALL is usually CD13 or CD33 positive. But myeloid specific MPO and CD117 is negative, which can help in distinguishing from mixed lineage acute leukemia. B-ALL with t(1,19) show CD34 negativity and positive for CD19,CD10, CD20. B-ALL with t(12,21) display a higher intensity of CD10 and HLADR with lower levels of the CD45, CD20 and CD34 [3]. Aberrant NK/T cell marker CD56 is also more common in t(12,21) cases. Over expression of CD22 is common in B-ALL with hyperdiploidy [4]. Flowcytometry not only can diagnose acute leukemia from peripheral blood/bone marrow, it can also detect leukemic cells in cerebrospinal fluid to detect central nervous system (CNS) involvement, to start intracranial CNS prophylaxis. It can also detect isolated CNS or extra medullary relapse as myeloid sarcoma by analyzing the cerebrospinal fluid or other body fluids or tissue.

Prognosis

There are many immonophenotypic markers which are associated with bad prognosis in acute leukemia. In acute myeloid leukemia, expression of CD7, CD9, CD11b, CD13, CD14, CD33, CD34, CD56, TdT are associated with bad prognosis [5-12]. In another study, co-expression of CD34 and HLA-DR shown to have an independent predictor of failure to achieve complete remission (CR) [13]. Another study described a more favorable prognosis in cases with blasts expressing panmyeloid markers: myeloperoxidase (MPO), CD13, CD33, CDw65 and CD117 [14]. Expression of CD2, CD34, and CD56 are associated with poor prognoses in APML. CD 2 expression is also associated with leukocytosis, hypogranular variant and higher chance of thrombosis [15]. CD20 expression in adult precursor B-lineage ALL is associated with a poor prognosis [16]. Bright CD45 expression is associated with bad prognosis with high chance of relapse in cases of B and T cell ALL [17]. Additionally, the flow cytometric DNA index

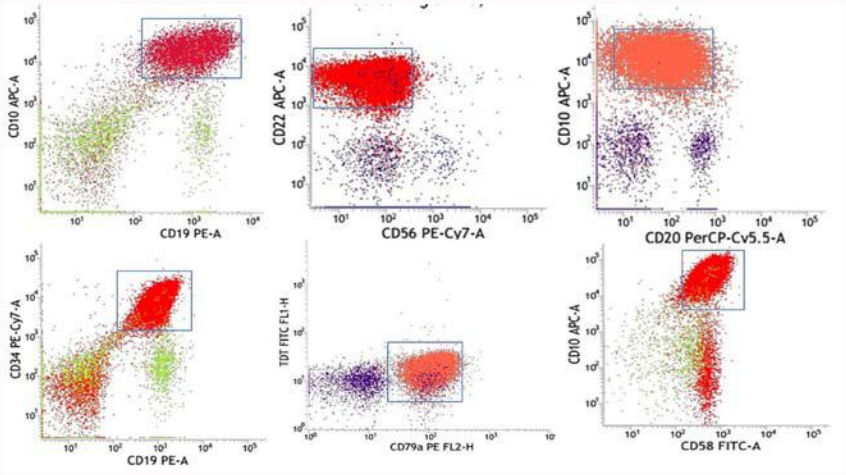

Figure 3: B ALL cases with positive B lineage CD markers (blasts are gated in square box).

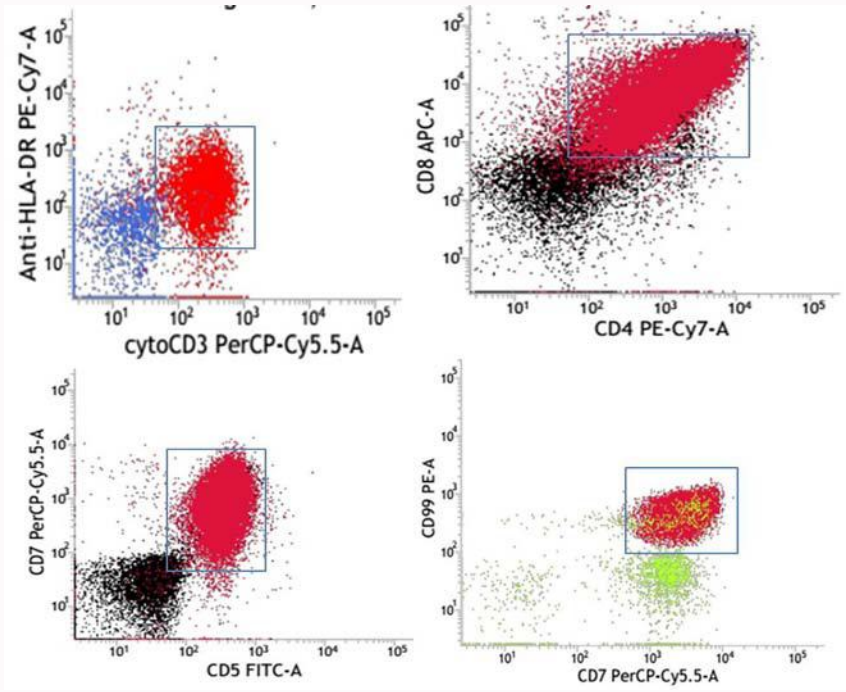

Figure 4: T ALL cases with positive T lineage CD markers (blasts are gated in square box).

(DI) can detect DNA ploidy which is a prognostic factor in ALL. In childhood ALL, a DI of ≥1.16 is associated with hyperdiploidy of >50 chromosomes which has a favorable outcome. On the other hand, a hypodiploid clone (<44 chromosomes) is associated with a poor prognosis [18].

Treatment

Therapeutic applications of monoclonal antibodys (MoAbs) in acute leukemia include immunologic techniques for purging malignant cells from autografts prior to transplantation, T-lymphocyte depletion from allografts as a strategy to reduce graft-versus-host disease and monitoring the timing and extent of leukapheresis in peripheral stem cell transplantation [19]. Therapy with MoAbs directed against leukemia surface antigens including CD19, CD20, CD22, and CD52 are an attractive targeted treatment approach, particularly beneficial where further intensification of chemotherapy is impossible, particularly when there is minimal overlapping toxicity. Anti CD20 monoclonal antibody, Rituximab has been incorporated into regimens for Burkitt-type leukemia/lymphoma such as dose-modified CODOX-M/IVAC (cyclophosphamide, vincristine, doxorubicin, and high dose methotrexate alternating with ifosfamide, etoposide, and high dose cytarabine) and dose-adjusted EPOCH (etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin) as high CD20 expression in these case with and potential synergy with chemotherapeutic agents, particularly given the successes observed with encouraging preliminary results [20,21]. Rituximab has also been incorporated into a modified hyper-CVAD regimen for adolescents and adults precursor B-cell ALL with de novo CD20 positivity [22]. Some authors suggest that, as CD20 antigen is not expressed by normal neurons or glial cells, Rituximab has been therefore been used intrathecally for the management of leptomeningeal infiltration in CD20 positive ALL [23]. Ofatumumab

which is a second-generation anti-CD20 monoclonal antibodyin is found effective in combination of hyperfractionated-CVAD (HCVAD) in pre-B CD20+ ALL [24]. Obinutuzumab is a novel glycoengineered type II CD20 monoclonal antibody that is superior to rituximab and ofatumumab in the induction of direct cell death, but warrants further investigation to use in B-ALL cases [25]. Monoclonal antibodies targeting CD19 (f SAR3419, SGN-CD19) and CD22 (Epratuzumab, Inotuzumab ozogamycin) are under evaluation in clinical trials of refractory-relapsed ALL. Alemtuzumab is a humanized monoclonal antibody against CD52 and can be used in patients with CD52-positive acute leukemia [26].

Follow up (Minimal Residual Disease (MRD)

Minimal Residual Disease (MRD) is defined as leukemic population undetectable by morphologic methods. In other words, MRD is a term used when there is evidence (immonophenotypic, molecular, or cytogenetic) leukemic cells remain in the bone marrow but there are insufficient cells to be detected by routine morphological examination [27]. It can predict early relapse and can also help in risk stratification in acute leukemia. Flowcytometry allows the detection of 1 leukemic cell among 10,000 normal cells (0.01%). The most common differential of neoplastic blasts is hematogones and regenerating blasts, which can be differentiated on flowcytometric immunophenotyping. Hematogones exhibit a well-defined spectrum of antigen expression as they mature: stage 1: The most immature hematogones express CD34, TdT, and slightly bright CD10 and CD20 negative, stage 2: As they mature, they lose CD34 and TdT expression and less bright for CD10, stage 3: Gradually express CD20 and dim surface immunoglobulin. Immonophenotypic detection of MRD in acute leukemia can be performed by defining aberrant marker expression, denoted as Leukemia-Associated Phenotypes (LAPs) on leukemic blasts at diagnosis. LAPs are immonophenotypic aberrancies defined as patterns of antigen expression on neoplastic cells that are different from those seen on normal hematopoetic cells. The common aberrancies in AML are asynchronous antigen expression as co-expression of CD34 and CD15; absence of lineage specific marker as CD13, CD33; overexpression or under expression of myeloid markers and expression of lymphoid associated markers as CD2, CD19, CD79a, CD7, CD10. The most frequent aberrancies in B-ALL are uniform positive expression of TdT and CD34, underexpression of CD45, overexpression of CD10 and CD58, underexpression of CD38, and underexpression of CD20. Asynchronous co-expression of CD34/CD10 with CD20/CD22 is also frequently observed. There may expression of myeloid-associated antigens as CD13/CD33/CD65/CD15/CD11b or very rarely T cell–associated antigens (CD2/CD4/CD5/CD7) [4]. In T-ALL, the common aberrancies are co-expression of CD4 and CD8; under expression of CD7,CD5; expression of stem cell/ myeloid markers as CD34, HLA-DR, CD13, CD33, CD117,CD65, CD15, CD11b etc. The aberrancies are detected by flowcytometry at the time of diagnosis and can be compared in follow up bone marrow samples to detect presence of residual leukemic cell and can predict an early relapse.

Conclusion

Despite of the increasing importance of molecular and genetic features in the sub-classification of acute leukemias, morphologic and immunophenotypic analysis remains the main modality to diagnose acute leukemia for initial evaluation and providing a rapid assessment to direct specific molecular genetic tests. Flowcytometric immunophenotyping may directly correlate with prognosis and in an era of novel agents may help in development of monoclonal antibodies to the tumor antigens. In addition to that, flowcytometry is the main stay of evaluating minimal residual disease, particularly in cases without any specific molecular signature.

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