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Review Article

Minimal residual disease detection using flow cytometry: Applications in acute leukemia

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ABSTRACT

Minimal residual disease (MRD) describes disease that can be diagnosed by methodologies other than conventional morphology, and includes molecular methods (like polymerase chain reaction (PCR)) or flow cytometry (FCM). Detection and monitoring of MRD is becoming the standard of care, considering its importance in predicting the treatment outcome. MRD aids in identifying high-risk patients and hence therapy can be intensified in them while deintensification of therapy can prevent long-term sequelae of chemotherapy in low-risk category. FCM is considered as a less labor-intensive and faster MRD technique as compared to PCR although it has its own share of disadvantages. Current immune-based methodologies for detection of MRD depend on establishing leukemia-associated aberrant immunophenotype (LAIP), at diagnosis or relapse and use this information at specified time points for detection of MRD, or apply a standardized panel of antibody combinations for all MRD cases, in a different-from-normal approach. This review highlights MRD detection by FCM and its application in acute leukemia.

Introduction

Remission in acute leukemia is considered when neoplastic cells percentage falls below 5% of marrow nucleated cells. Cases of acute leukemia taken to be in remission by above-mentioned criteria may still harbor a large number (up to 10^{10}) of undetectable malignant cells.[1] This has often led to disease relapse in cases presumed to be in remission according to above-mentioned parameter. Minimal residual disease (MRD) describes disease that can be diagnosed by methodologies other than conventional morphology, and includes molecular methods (like polymerase chain reaction (PCR)) or flow cytometry (FCM). It can further be categorized as immunologic MRD (detected by FCM) or molecular MRD (detected by PCR).[2] Around 40% of patients with AML have no genetic markers suitable for PCR monitoring. For those, FCM remains the only option. For others, a combination of the two is ideal and gives maximum information.

Over a period of 25 years, several PCR-based and flow cytometric MRD technologies have stepwise developed into routinely applicable MRD tools, particularly because of

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long-term international collaboration with open exchange of knowledge and experience and collaborative experiments. However, each technique is associated with their inherent advantages and disadvantages.[3–10] Sensitive techniques to detect MRD are expected to give accurate estimation of burden of leukemia and formulation of better therapeutic protocols. MRD detection on FCM platform requires detection of immune phenotype markers with selective positive expression on leukemic cells vis-a-vis negative expression on normal cells of hematopoietic lineage. Such selectivity ensures a very high detection rate (1 leukemic/10,000 normal hematopoietic cells of bone marrow), and up to 2/3 of acute leukemia patients are expected to be benefitted out of it. Also, according to various studies, MRD levels are strongly associated with treatment outcome and clinical remission.[11,12]

Biology and treatment of acute leukemia

In present day scenario, better survival rates for acute leukemia patients treated with intensified regimes have emerged, partly due to much improved supportive care. Current multiagent regimens can offer cure in majority (up to 80%) of pediatric acute lymphoblastic leukemia (ALL) patients.[13,14] Pediatric acute myeloid leukemia (AML) and adult AML/ALL have also shown encouraging trends with multiagent regimens, albeit to a lesser degree. However, intensified chemotherapy is itself fraught with the risk of developing secondary malignancies, cardiomyopathy, and neuropsychiatric manifestations.[15,16] Hence, there was a requirement for identification of the subset of patients with minimal disease burden in them so that the late sequelae of high dose intensified chemotherapy can be avoided. Similarly, the patients with resistant disease below the resolution of morphology can be given intensified therapy or stem cell transplant at an early date to enhance the disease-free survival.

Response to various therapeutic regimes is also affected by the heterogeneous biologic features of each subgroup of leukemia. For example, most often than not, stem cell transplant is the only curative option for pediatric ALL with the *t*(9; 22) translocation or *MLL* gene rearrangements due to less than adequate response to chemotherapeutic agents. Similarly, response to chemotherapy has been quite well in pediatric patients with leukemic cells containing 51–65 chromosomes or rearrangements of *TEL*. In cases of AML, a similar better response to chemotherapy has been seen in cases with 16q22 translocations, *t*(15; 17)/*t*(8; 21). Hence, biologic features and certain clinical parameters (age, leukocyte count, etc.) are used for formulation of therapeutic protocols, in view of a significant correlation seen between them and the clinical outcome in acute leukemia patients.[17]

However, any of the current clinical or biologic features purported is far from being ideal. On one hand, we have seen relapses in patients with 'good risk' features, and on the other hand, unnecessary high intensity treatment has been provided in certain cases. Such inadvertent events may be avoided by MRD studies, as MRD studies during clinical remission are aimed at improving the total leukemic cells burden estimation. Appropriate treatment stratification can be done if one has this MRD information, as it can give an indication to the sensitivity to chemotherapeutic agents and disease aggressiveness.

MRD and its clinical applications

Classically, there are three different approaches used in monitoring MRD which include multiparameter flow cytometric immunophenotyping (FCM), real-time quantitative polymerase chain reaction (RQ-PCR)-based detection of clonal immunoglobulin (Ig) and T-cell receptor (TCR) gene rearrangements and RQ-PCR-based detection of fusion transcripts or breakpoints or aberrant/overexpressed genes. The principles and characteristics and the pros and cons of these MRD techniques are summarized below (Table 1).

Immunologic detection of MRD by FCM

Identification of leukemic cells

Flow cytometry was explored as a less labor-intensive and faster MRD technique, when 4- and 6-color cytometers became available in 1998–2002. These multicolor approaches followed classical concepts with emphasis on the detection of aberrant immunophenotypes in the ''empty spaces'' (not overlapping with normal leukocytes) in 2-dimensional dot plots.[3] However, relative nonavailability of monoclonal antibodies to markers on blasts, which were also shared by normal hematopoietic cells had hampered the earlier attempts to study MRD immunologically. A point in case is the concomitant expression of terminal deoxynucleotidyltransferase (TdT) and cluster of differentiation (CD)10 on most leukemic lymphoblast as well as normal B cell precursors (hematogones). Hematogones are seen in plenty in pediatric bone marrow and regenerating marrow following chemotherapy or hematopoietic stem cell grafting.[18] Leukemic cells scattered in bone marrow cannot be detected by using expression of TdT and CD10 in isolation, even though abnormal levels of these markers have been found in some leukemic cases. However, certain differences do exist regarding the expression of these immunophenotypic markers on leukemic cells vis-a-vis normal cells. The abnormal expression of immunophenotypic markers distinguishes the leukemic cells termed as leukemia-associated aberrant immunophenotype (LAIP), which could be cross-lineage antigen expression, maturational asynchrony, and under/overexpression or loss of particular antigen. There may be quantitative or qualitative or both types of the antigenic expression differences among normal progenitor and leukemic cells. Combination of immunophenotypes selectively expressed by blasts with very rare expression on normal marrow cells constitutes the qualitative differences. Notable examples are CD34/CD19/CD21 and CD34/CD56 expression in some cases of B-ALL and AML, respectively. These immunophenotypic combinations, even if they are expressed on normal cells (which is extremely rare), have a very weak expression in comparison to leukemic blasts cells expression levels. T-ALL cells also express a unique combination CD3/TdT, which apart from T cells developing in the thymus, is almost never found on normal hematopoietic cells.