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Expression of the C-KIT Molecule in Acute Myeloid Leukemias: Implications of the Immunophenotypes CD117 and CD15 in the Detection of Minimal Residual Disease

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ABSTRACT

Objectives: Study of the c-kit proto-oncogene (CD117) may be of help for the identification of phenotypic profiles that are absent or present at very low frequencies on normal human blast cells and therefore might be of great value for the detection of leukemic cells displaying such immunophenotypes in patients in complete remission.

Design and methods: Ninety patients with acute myeloid leukemias, diagnosed according to FAB criteria and immunological marker studies, were studied for the dual expression on blast cells of the CD117/CD15 immunophenotype coexpression by direct immunofluorescence assay using dual staining combination flow cytometry.

Results: In 69/90 acute myeloid leukemia patients analyzed (77%), blast cells expressed the CD117 antigen. Moreover, in 38 of them (42% of acute myeloid leukemia cases), leukemic blasts coexpressed the CD117 and CD15 antigens. There was no significant correlation between the FAB classification and the CD117 and CD15 expression in acute myeloid leukemia cases.

Conclusions: These results suggest that immunological methods for the detection of MRD based on the existence of aberrant phenotypes could be used in the majority of AML patients. This phenotype CD117/CD15, present in acute myeloid leukemia cases at a relatively high frequency (42%), represents an aberrant phenotype, because it was not detected on normal human blast cells, suggesting that the use of these combinations of monoclonal antibodies could be of help in detecting residual leukemic blasts among normal blast cells. The use of the CD117 antigen in different monoclonal antibodies combinations may be of great help for the detection of minimal residual disease in a high proportion of acute myeloid leukemia cases, especially in those patients displaying the CD117+/CD15+ immunophenotype, because cells coexpressing both antigens in normal blasts, if present, are at very low frequencies. The simultaneous assessment of two or more markers in single cells has facilitated the identification of multiple aberrant phenotypes which should permit the detection of impending relapses prior to clinical manifestations; MRD testing in patients with AML in clinical remission is a potentially useful tool for assessing the risk of relapse and guiding therapeutic decisions. The detection of MRD before overt relapse may provide information for early intervention, while definitive recognition of normal recovering blasts may prevent unnecessary treatment.

Key Words: *C-kit - CD117- CD15 - AML - MRD - Flow cytometry.*

INTRODUCTION

Immunophenotyping is an essential method for diagnosis and classification of AML and its extensive use could identify blast cell subpopulations with aberrant phenotypes rarely seen in normal myelopoiesis. The aberrant phenotypes have been correlated with clinical, morphological and prognostic features, but their occurrence in AML differs in the various studies. Aberrant phenotypes are present in the great majority of AML cases, asynchronous antigen expression being the most frequent example and the CD117+ CD15+ phenotype shows a relevant association with clinical prognosis **[3]**.

Complete remission (CR) rates as high as 70% to 80% have been reported in patients with acute myeloid leukemia (AML), but approximately 60% to 70% of patients will eventually have relapse due to the persistence of residual leukemic cells surviving after chemotherapy. The persistence of residual malignant cells below the threshold of conventional morphologic findings-minimal residual disease (MRD)-may identify patients at a higher risk of relapse **[40]**.

Direct immunofluorescence flow cytometry (DIF) ad polymerase chain reaction (PCR) are the most commonly used techniques for detecting MRD in patients with AML. Although PCR studies in patients with AML have demonstrated the value of this approach for monitoring MRD, however, only a fraction of patients with AML have fusion transcripts suitable for PCR assays [20].

Immunophenotyping of bone marrow specimens with AML may be performed by flow cytometry or immunohistochemical techniques. Some markers (CD117 and CD15) are available for both techniques, however, immunodetection of CD117 and CD15 had a higher sensitivity by flow cytometric analysis than by immunohistochemical analysis, so, CD117 and CD15 are better detected by flow cytometric analysis [9].

MRD monitoring with flow cytometry relies on the idea that AML cells frequently show aberrant or leukemia-associated phenotypes resulting from asynchronous antigen expression, cross-lineage antigen expression, antigen overexpression and aberrant light-scatter properties [26].

Multidimensional flow cytometry allows the discrimination of antigens expressed on normal and malignant cells and can detect small numbers of cancer cells in bone marrow or peripheral blood specimens [35]. The detection of cells with unusual phenotype may reflect residual AML cells and their increase may predict relapse [32]. MRD testing in patients with AML in clinical remission is a potentially useful tool for assessing the risk of relapse and guiding therapeutic decisions [37].

Immunophenotypical studies of AML have shown that leukemic cells are heterogenous not only among different individuals but also within each patient. Until now, immunological studies on MRD detection have been based on the follow-up of the predominant blast cell population at diagnosis. However, relapses can occur on less represented subpopulation which in turn is highly resistant to chemotherapy [23].

C-kit monoclonal antibodies identify an extracellular epitope of the proto-oncogene and class III receptor tyrosine kinase c-kit, which has been shown to be the receptor for the stem cell factor or steel factor and recently clustered as CD117. This antigen is expressed by between 1% and 4% of normal human bone marrow mononuclear cells, including multipotent, erythroid and myeloid committed progenitor cells [22].

Certain combinations of differentiation antigens are expressed on leukemic blasts and are absent or extremely rare among normal progenitros. These combinations include the $CD117^{+}/CD15^{+}$ immunophenotype, because cells coexpressing both antigens in normal bone marrow, if present, are at very low frequencies. Thus, double marker immunofluorescence assay is a leukemia-specific technique that can be applied in AML for detecting MRD [7].

Many studies have demonstrated that immunologic evaluation of residual disease contributes to the prediction of outcome in AML patients in morphologic CR and that the immunophenotypical assessment of remission is more accurate than the morphologic examination [26].

Accurate determination of MRD may have profound impact in the clinical management practices of patients with hematological malignancies. The definition and the clinical importance of MRD at different stages of treatment is the first goal to be pursued by many studies, the success of which depends on the close collaboration between laboratory investigators and clinicians [6].

The aim of the present study is to analyze the incidence of AML cases displaying the dual blast cell immunophenotype CD117/CD15 coexpression, since even if minimal, it can be responsible for the relapse [23]. For this purpose, we have prospectively investigated the immunophenotype in blast cells from 90 de novo, relapsed and in remission AML patients using double monoclonal antibodies in dual staining combinations analyzed by flow cytometry.

PATIENTS AND METHODS

This study included 90 patients with AML (45 adults and 45 pediatrics). Patients were diagnosed as AML on the basis of blast counts (≥ 20%), myeloid markers by immunophenotyping studies and morphologic and cytochemical examination and they were classified according to the FAB criteria.

Patients were collected from the Clinical

Oncology, Internal Medicine and Pediatrics Departments of Beni Soeif and Kasr El-Aini University Hospitals and National Cancer Institute.

Patients were selected as one third of them was diagnosed as de novo AML, other third of patients were in complete clinical and morphological remission and the last third of AML patients were in relapse.

The age of adult patients ranged between 37 to 69 years with a mean of 54.73±9.12 and a median of 55.5 years. They were 25 males and 20 females with a male to female ratio 1.25:1. The age of the pediatric patients ranged between 7 to 18 years with a mean of 13.39±2.19 and a median of 15.25 years. They were 24 males and 21 females, with a male to female ratio 1.1:1.

All patients were subjected to the following:

* Thorough history taking.

* Clinical examination both general and local.

* Laboratory investigations including:

- • Routine laboratory investigations:
  - Complete hemogram.
  - Bone marrow aspiration.
  - Cytochemical studies and
  - Immunophenotyping examination.
- • Specific laboratory investigations:
  - Study of the dual expression of CD117 and CD15 on bone marrow blast cells by immunophenotyping using flow cytometry.

Immunophenotyping was performed by dual-colour flow cytometric analysis on bone marrow aspirates collected on EDTA from de novo, relapsed and in remission AML patients, using the whole-blood lysis technique [32].

Cells were stained with phycoerythrin (PE)-conjugated monoclonal antibody (CD117-PE) and fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody (CD15-FITC) (Becton Dickinson Company). Stained cells were analyzed using FACScan (Becton Dickinson & Co.).

Ten hematologically normal bone marrow aspirates were obtained from allogenic bone marrow transplant donors, serving as controls.

The induction treatment combined cystarabine (100 mg/m^2 of body surface area given intravenously on days 1-10), etoposide (100 mg/m^2, given on days 1-5) and on days 1,3,5, either duanarubicin (50 mg/m^2), mitoxantrone (12 mg/m^2) or idarubicin (10 mg/m^2). As consolidation, patients received cystarabine (300 mg/m^2 every 12 hours on days 1-6) and the same anthracyclin given in the induction (on days 4-6).

RESULTS

Ninety patients were included in this study, forty-five of them were adults and the other forty-five patients were in the pediatric age group, thirty patients out of ninety were diagnosed as de novo AML, thirty patients were in complete clinical and morphological remission and the last thirty patients were in relapse.

Patients were diagnosed as de novo AML on the basis of blast cell counts (≥ 20%) in the bone marrow, myeloid markers by immunophenotyping studies (CD13 and CD33) and morphological and cytochemical examination (peroxidase positive blasts) and they were classified according to the FAB criteria. Patients were diagnosed as AML in remission having less than 5% of blasts in the bone marrow. The disease was considered in relapse when more than 10% of blasts are seen in the bone marrow [5,6].

The age of adult patients ranged between 37 to 69 years with a mean of 54.73±9.12 and a median of 55.5 years. They were 25 males and 20 females with a male to female ratio 1.25/1. The age of the pediatric patients ranged between 7 to 18 years with a mean of 13.39±2.19 and a median of 15.25 years. They were 24 males and 21 females, with a male to female ratio 1.1/1.

According to the FAB classification, M2 was the most predominant category of AML as it was observed in 26 out of 90 patients, followed by M4 in 23 patients, M5 in 22 patients, M3 in 15 patients, 3 patients in M1 and only one patient in M0.

In 69/90 AML patients analyzed (77%), blast cells expressed the CD117 antigen (mean percentage of positive cells was 89.8±27.6%).

Moreover, in 38 of them (42% of AML cases), leukemic cells coexpressed the CD117 and CD15 antigens (mean percentage of double-positive cells was 19.7±3.5%).

The mean number of CD117+ cells identified in normal bone marrow samples was 0.54±0.23%. CD117+ cells coexpressing the CD15 antigen were not detected.

Adult patients with AML expressed the CD117 antigen in 34 patient out of 45 (75.5%) while 19 adult patients out of 45 coexpressed both the CD117 and the CD15 antigens (42%). Expression of c-kit molecule CD117 in the pediatric age group was observed in 35 out of 45 patients (77.7%), while both antigens CD117 and CD15 were coexpressed in 19 patient out of 45 pediatric patients (42%).

Table (1) shows the characteristics of AML patients included in this study.

Table (2) shows the expression of CD117 antigen in AML patients.

Table (3) shows the coexpression of CD117/CD15 antigens in AML patients.

Fig. (1) shows expression of CD117 antigen in AML patient.

Fig. (2) shows coexpression of CD117/CD15 in de novo AML patient.

Fig. (3) shows coexpression of CD117/CD15 in AML patient in relapse.

Fig. (4) shows coexpression of CD117/CD15 in AML patient in remission.

Table (1): The characteristics of AML patients included in this study.

Characteristic	Value**
Total number:	90
Adults	45
Pediatrics	45
Clinical status:	
De novo	30
In remission	30
In relapse	30
Sex:	
Males	49
Females	41
Ratio	1.2
Age (Y) (mean ± SD):	
Adults	54.73±9.12
Peditrics	13.39±2.19
FAB classification:	
M0	1
M1	3
M2	26
M3	15
M4	23
M5	22

** Unless otherwise specified, Value = Number of patients.

Table (2): The expression of CD117 antigen in AML patients.

Morphological & clinical status	Adult patients No=45	Pediatric patients No=45
De novo	13/15 (87%)	12/15 (80%)
in remission	8/15 (53%)	9/15 (60%)
In relapse	13/15 (87%)	14/15 (93%)
	34/45	35/45
	69/90 (77%)	

Table (3): Expression of CD117/CD15 phenotype in AML patients.

Morphological & clinical status	Adult patients No=45	Pediatric patients No=45
De novo	6/15 (40%)	7/15 (47%)
in remission	3/15 (20%)	3/15 (20%)
In relapse	10/15 (60%)	9/15 (60%)
	19/45	19/45
	38/90 (42%)	

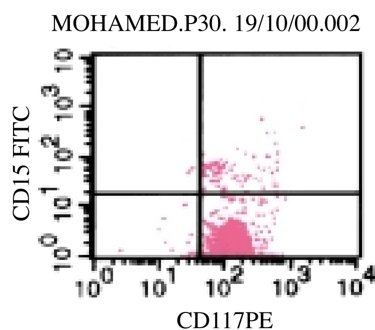

Fig. (1): Expression of CD117 antigen in AML patient.

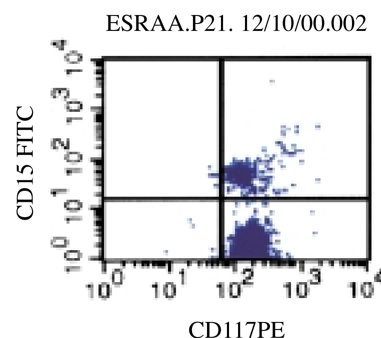

Fig. (2): Expression of CD117/CD15 in de novo AML patient.

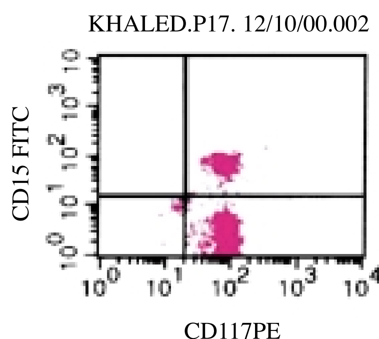

Fig. (3): Expression of CD117/CD15 in AML patient in relapse.

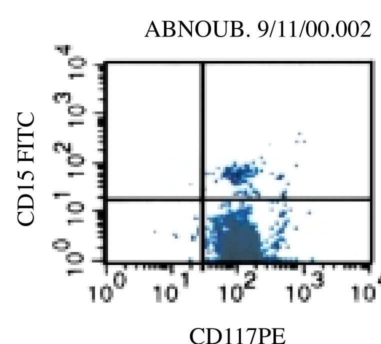

Fig. (4): Expression of CD117/CD15 in AML patient in remission.

DISCUSSION

A high complete remission rate is currently achieved in patients with AML. However, many patients eventually relapse due to the persistence of low numbers of residual leukemic cells that are undetectable by conventional cytomorphologic criteria (MRD). Among the techniques suitable for MRD detection, immunophenotyping and polymerase chain reaction analysis of leukemia-specific sequences are the most commonly used. Information concerning the value of MRD investigations is significantly lower in AML than it is in ALL. Moreover, most available information on MRD in AML is based on molecular detection of chromosomal translocations. Regarding immunophenotypic studies, although there are no leukemia-specific antigens that can be used as target markers for MRD detection, many studies, have shown that leukemic cells frequently display aberrant or uncommon phenotypic features that allow their distinction from normal cells. For AML, the most relevant types of aberrant forms include (1) asynchronous antigen expression (expression of a combination of myeloid-associated antigens which is not found in the normal myeloid differentiation such as the coexpression of CD117 and CD15 antigens); (2) cross-lineage antigen expression (coexpression of lymphoid-associated markers on myeloid blast cells); (3) antigen overexpression (abnormally high expression of a certain antigen on blast cells) and (4) myeloid cells displaying aberrant light-scatter properties. Asynchronous antigen expression of myeloid differentiation antigens occurs in the majority of patients with AML **[3,26, 32,40]**.

Initially, studies on immunophenotypical detection of MRD have been based on double antigen stainings analyzed by fluorescence microscope. More recent data show that flow cytometry allows a more precise detection of phenotypic aberrations. Moreover, previous MRD studies have been based on the phenotypic characteristics of the predominant blast cell population, ignoring the existence of two or more phenotypically different blast cell subsets at diagnosis in a high proportion of AML cases

and not taking into account that relapse could be due to a minor blast cell subset that in turn could be highly resistant to chemotherapy, so the aim of many studies is to define within leukemic cells those phenotypes that are absent or extremely infrequent in normal bone marrow samples **[10-13,26]**.

Analysis of AML surface phenotypes has been complicated by the heterogeneity associated with this disease. Many researches have studied the coexpression of markers rarely or never appearing simultaneously in normal hematopoietic differentiation and many analyses have selected the major coexpression pattern; the CD117+/CD15+ (up to 99% of the blasts in some cases). The detection of cells with unusual phenotype may reflect residual AML cells, and their increase may predict relapse **[28, 31 & 32]**.

Morphologic analysis of bone marrow in patients with AML is very useful for defining a subgroup of patients that do not achieve complete remission and display a very poor prognosis. However, among those that achieve complete remission, a high incidence of relapses persists and these cannot be predicted using conventional morphology. Therefore it is necessary to search for alternative approaches that would allow the detection of lower levels of residual leukemic cells, with a potentially higher value for predicting relapse. In many studies it is shown that the immunophenotypic investigation of MRD is useful to predict outcome in AML patients in morphologic complete remission and perhaps the term immunologic remission could replace that of morphologic complete remission or at least be complementary to it. The number of residual cells displaying leukemic-associated phenotypes correlates with the probability of relapse, the remission duration and the predicted survival **[26,36,39]**.

Immunophenotypical studies of AML have shown that leukemic cells are heterogenous not only among different individuals but also within each patient. Accordingly, positive and negative cells for a certain antigen are frequently detected among AML patients. This finding could suggest the existence of more than one blast cell population within the leukemic clone. Although it is probably not relevant for diagnostic purposes, the identification of the possible blast cell subpopulations based on their immunophenotype may be critical for the investigation of residual leukemic cells. Until now, immunological studies on MRD detection have been based on the follow-up of the predominant blast cell population at diagnosis. However, relapses can occur on a less represented subpopulation which in turn is highly resistant to chemotherapy. Thus, the aim of many studies applied on AML patients is to explore the incidence of leukemic cell subpopulations and to analyze whether the heterogeneity of blast cells in AML is either due to a certain degree of differentiation within the leukemic clone or related to the existence of phenotypically different leukemic subsets. For that purpose, a dual marker flow cytometry analysis is used in many researches in order to simultaneously assess the expression of two antigens on the leukemic cells **[23,41]**.

Despite aggressive induction chemotherapy, the majority of patients with AML experience leukemic relapses that cannot be predicted with current laboratory techniques. Dual-staining immunofluorescence, a quantitative assay that permits the identification of rare residual leukemic cells during clinical remission, was used for its ability to accurately predict leukemic relapse in patients treated for AML. Among patients who did not receive an allogeneic bone marrow transplant during the first CR, early bone marrow assessment with the dual marker staining flow cytometric assay identifies patients likely to experience relapse **[35,42]**.

The purpose of this study was to identify the leukemia-associated phenotype; the CD117/CD15 by flow cytometry and to use this phenotype for detecting MRD; residual malignant clone among normal bone marrow cells; thus predicting a subsequent impending leukemic relapse. The occurrence of leukemic features was demonstrated at diagnosis in a number of patients and it was investigated whether such immunophenotype may exist in remission or in relapse in other subsets of patients, to define within leukemic cells that phenotype that is absent or extremely infrequent in normal BM samples **[7,26,27]**.

The murine monoclonal antibody (CD117) identifies a transmembrane tyrosine kinase receptor encoded by the human c-kit protooncogene **[39]**. The c-kit gene product (CD117) is known to be expressed by a variety of normal human tissue cell types, including immature myeloid cells **[1]**. The c-kit receptor (CD117), a specific marker for the myeloid lineage hemato-

poietic progenitor cells which is expressed early during hematopoietic differentiation and its ligand stem cell factor (SCF) play an important role in the development, differentiation and survival of normal and malignant hematopoietic cells [19,34,39]. Patients with AML need to be tested for the CD117 (c-kit) expression to establish whether the expression of c-kit may define AML subgroups of prognostic significance [39].

In the present study, expression of the c-kit (CD117) molecule among patients with AML was observed in 69 out of 90 (77%) of patients, 34 out of 45 patients (75.5%) were adults and 35 patient out of 45 (77.7%) were pediatric AML patients. Most of the CD117+ cells coexpressed the myeloid associated antigen CD15 which was found to be expressed in 38 out of 90 AML patients (42%), being expressed in 19 out of 45 patients in either adult or pediatric AML patients. The mean percentage of positive CD117 cells was 89.8±27.6%, while the mean percentage of double-positive cells for CD117/ CD15 antigens was 19.7±3.5%. The mean number of CD117+ cells identified in normal bone marrow samples was 0.54±0.23%. Interestingly, CD117+ cells coexpressing the CD15 antigen were not detected in normal bone marrow. There was no correlation between the FAB classification of AML and the CD117 and CD15 antigen expression in AML cases studied. The dual expression of both CD117 and CD15 antigens in patients in complete clinical and morphological remission in 6/30 (20% of studied AML patients) suggests that the use of these combinations of monoclonal antibodies could be of help in detecting residual leukemic blasts among normal bone marrow cells.

Venditti and colleagues in (2000) denoted that direct immunofluorescence flow cytometry and polymerase chain reaction are the most commonly used techniques for detecting MRD in patients with AML. They noted that although PCR studies in patients with AML have demonstrated the value of this approach for monitoring MRD, however, only a fraction of patients with AML have fusion transcript suitable for PCR assays [40].

Dunphy and coworkers in (2001) stated that immunophenotyping of bone marrow specimens with AML may be performed by flow cytometry or immunohistochemical techniques. Some markers (CD117 and CD15) are available for both techniques, however, immunodetection of CD117 and CD15 had a higher sensitivity by flowcytometric analysis than by immunohistochemical analysis, so CD117 and CD15 are better detected by flowcytometric analysis. They observed that there was no correlation of the FAB subtype of AML and the CD117 and CD15 expression in their study on AML patients [9].

Our findings are in credence with the study of Macedo et al. (1995b), where from the 71 AML cases included in their study, 53 of them (75%) displayed reactivity for the CD117 antigen, 34 of these cases (48%) coexpressed the CD15 antigen. The mean percentage of positive CD117 cells was 85.7±29.0%, while the mean percentage of double-positive cells for both CD117/CD15 antigen coexpression was 37.1±3.6%. The mean number of CD117+ cells identified in normal bone marrow samples was 0.56±0.24%, while CD117+ cells coexpressing the CD15 antigen were not detected. They denoted that the definition of a phenotype as aberrant must be based on previous comprehensive studies on normal cell differentiation, and they confirmed that preliminary reports have shown that the CD117 antigen is coexpressed in 80% of de novo AML cases [22].

They suggested that this phenotype CD117/ CD15, present in AML cases at a relatively high frequency (48%), represents an aberrant phenotype, because it was not detected in normal human bone marrow. They stated that the use of these combinations of monoclonal antibodies could be of help in detecting residual leukemic blasts among normal bone marrow cells. They concluded that this type of analysis may be of help, not only to gain insight into the normal hematopoietic differentiation but also for the identification of phenotypic profiles that are absent or present at very low frequencies (< 0.01%) in normal human bone marrow and therefore might be of great value for the detection of leukemic cells displaying such immunophenotypes in patients in complete remission [22].

They indicated that simultaneous assessment of two or more markers in single cells has facilitated the identification of multiple aberrant phenotypes which ideally should permit the detection of residual leukemic cells and the prediction of impending relapses prior to clinical manifestations. In summary, they confirmed that the use of the CD117 antigen in different

monoclonal antibodies combinations may be of great help for the detection of minimal residual disease in a high proportion of AML cases, especially in those patients displaying the CD117+/CD15+ phenotype, because cells coexpressing both antigens in normal bone marrow, if present, are at very low frequencies **[22]**.

Cascavilla et al. (1998) noted that the CD117 molecule is an antigen more frequently found on early normal and leukemic hematopoietic cells, but correlation with the FAB subtypes is still not well established. They found that CD117 antigen was expressed in 74% of AML patients studied. They did not notice significant correlation between FAB classification and CD117 and CD15 expression, the same finding as in our study. They concluded that the CD117 antigen shows a high specificity for AML independently upon FAB classification and represents a reliable marker characterizing the prognostic degree of the myeloid blasts **[8]**. Khalidi et al. (1998) also did not notice significant correlation between the FAB classification and the CD117 and CD15 expressed in AML patients **[17]**.

Mateu and associates in (1999) denoted that the c-kit proto-oncogene encodes a 145 kd tyrosine kinase transmembrane receptor, which plays a key role in hematopoiesis. They stated that the c-kit has been classified as CD117 and it is especially useful in the prognostic outcome in AML. They indicated that CD117 expression was detected in 91% of AML cases in their study and they concluded that CD117 should be included on a routine basis for the immunophenotypic studies of AML **[25]**.

Schwartz and colleagues in (1999) noted that expression of the c-kit receptor was present in 63% of all AML patients investigated. They indicated that analysis of the c-kit receptor expression may help to identify phenotypically aberrant AML cases **[33]**.

Hiass and coworkers in (1999) stated that the increased CD15 expression on myeloid cells, together with the simultaneous expression of CD117 antigen in cases of AML, is suggestive of aberrant CD117+ CD15+ cells, which may indicate the leukemic origin of the maturing myeloid cells **[15]**.

Uaskan et al. (2000) indicated that the CD117 protein is expressed by the primitive CD34 positive hematopoietic stem cells and also demonstrated on the blasts of 30-100% of AML cases but rarely on lymphoblasts. They confirmed that CD117 molecule seemed to be a more specific marker for leukemia of myeloid origin being demonstrated on more than 5% of blasts in 60 out of 73 cases (82%) of AML patients in their study **[38]**.

Bahia et al. (2001) denoted that aberrant phenotypes were found in 88.6% of AML cases studied. In their study, asynchronous antigen expression was present in 82.4% of AML patients. They stated that among the cases of asynchronous antigen expression, the most frequent phenotype was CD117+ alone or in association with CD15+, corresponding to 67.6% and 50% of AML cases, respectively. They observed that asynchronous antigen expression correlates with clinical, morphological and prognostic features and that the CD117+ CD15+ aberrant phenotype correlates significantly with clinical status and prognostic expectations. They concluded that aberrant phenotypes are present in the great majority cases of AML, asynchronous antigen expression being the most frequent example; and that the CD117+ CD15+ phenotype shows a relevant association with clinical prognosis **[3]**.

Willman (1999) stated that, traditionally, AML has been diagnosed and classified based on the morphologic and cytochemical criteria of the FAB classification system. However, more recent studies has demonstrated that the immunophenotypic, cytogenetic and molecular genetic abnormalities consistently associated with most forms of AML confer the most important prognostic information. Each broad category of de novo AML that arise in infants and children, young adults and the elderly, or, AML arising subsequent to antecedent MDS or prior exposure to chemotherapy, radiotherapy, or environmental agents is characterized by distinct immunophenotypic, cytogenetic and molecular genetic abnormalities. He denoted that over the past 15 years, a number of recurrent immunophenotypic, cytogenetic and molecular genetic abnormalities have been cloned and characterized yielding valuable insights into the mechanisms of leukemogenesis and powerful molecular tools for precise diagnosis and monitoring of MRD. He concluded that as immunophenotypic, cytogenetic and molecular genetic abnormalities characterizing cells is now considered cru-

cial for both diagnostic and therapeutic decision making. It is essential to have a working knowledge of the immunophenotypic, cytogenetic and molecular genetic abnormalities of AML [43].

Negrin (1998) denoted that a major limitation in the treatment of malignant hematological disorders is the accurate and sensitive detection of MRD. He noted that it is clear that routine clinical and morphologic studies are extremely important, however, lacking in sensitivity. He added that, clearly, the goal of MRD detection is to make individual treatment decisions such that those patients who require more aggressive approaches are treated promptly and to avoid toxic and expensive treatments for those patients who do not require them. He concluded that to reach this goal, two milestones are required. The first is to develop and standardize sensitive and specific tools to detect MRD. The second is to determine whether these tools in fact predict clinical outcome [29].

Baer (1998) and Engel and associates (1999) indicated that the use of highly sensitive and specific assays to detect MRD in patients with acute leukemia should provide a basis for optimizing and maximizing each patient's chance of cure. They stated that strategies for detecting MRD are based on biologic properties that allow acute leukemia to be distinguished from normal marrow cells and that techniques used to detect MRD in acute leukemia include flow cytometry and polymerase chain reaction. Flow cytometry is better used to investigate the frequency and presence of MRD in AML [2,10].

The combination of flow cytometry and multiple stainings with monoclonal antibodies has allowed a better characterization of both leukemic and normal hematopoietic cell populations. The analysis of normal and leukemic hematopoietic precursor cells contributes to both the knowledge of normal cell differentiation and to the identification of aberrant phenotypes for MRD investigations [24].

Piedras and Cairdenas (1997) and Nagler et al. (1999) stated that flowcytometric detection of MRD helps the identification of immunophenotypic combinations expressed on leukemic cells but not on normal cells and that cannot be detected by conventional laboratory techniques, thus helps the selection of appropriate therapeutic strategies. They denoted that double-staining combinations may show diagnostically useful deviations from normality [28,30].

The existence of leukemia-associated phenotypes has been suggested to be a valuable tool for the detection of MRD in AML patients, as they would allow to distinguish leukemic blast cells from normal hematopoietic progenitors. Asynchronous antigen expression was detected in the majority of AML patients, with the CD117+ CD15+ phenotype showing a high frequency. Immunological methods for the detection of MRD based on the existence of aberrant phenotypes could be used in the majority of AML patients [21].

Many studies confirm the existence of phenotypically different blast cell subpopulations in most AML patients, and point to the need to perform a careful multiparametric analysis for their correct characterization. In addition, many researches suggest that MRD detection based on immunological methods should be based on the phenotypes of all leukemic cells subpopulations and not only on the most represented blast cell subset, or the original phenotype presented at diagnosis [23].

However, it is likely that the immunologic analysis described in this report will remain useful for patient monitoring due to its speed and relative simplicity. We found that on the basis of the observations on AML presented above, controlled trials are to be justified for retreating patients in early relapse as defined by CD117/CD15 double labelling [7].

These data indicate the prognostic significance of residual AML cells demonstrated by dual marker staining flow cytometry. If these data are put into consideration, the presence of these aberrant cells my indicate more aggressive treatment, and serve to redefine remission in AML [32].

In conclusion, accurate determination of MRD may have profound impact in the clinical management practices of patients with AML. The definition of the clinical importance of MRD at different stages of treatment is the first goal to be pursued by many studies, the success of which depends on the close collaboration between laboratory investigators and clinicians [6].

From the point of morphologic remission until overt relapse, the level of leukemic cells in the body is largely unknown, resulting in clini-

cal management strategies that do not discriminate among patients by their levels of MRD. Thus, patients with minimal leukemic cells are treated on the same regimen as those with much lower levels or, perhaps, with no leukemia at all **[6]**.

Miguel and coworkers in (1997) and Fujisawa and colleagues in (1998) stated that the number of residual leukemic cells at the end of induction or intensification therapy is the most important prognostic factor for prediction of remission. They confirmed that overall, immunophenotypical investigation of MRD strongly predicts outcome in patients with AML and that the number of residual leukemic cells correlates with prognosis and clinical outcome. In summary, they concluded that residual leukemic cells counts in the bone marrow at the end point of induction therapy is a valuable prognostic factor for patients receiving induction therapy for AML **[14,26]**.

MRD testing in patients with AML in clinical remission is a potentially useful tool for assessing the risk of relapse and guiding therapeutic decisions **[40]**. The detection of MRD before overt relapse may provide information for early intervention, while definitive recognition of normal recovering blasts may prevent unnecessary treatment **[41]**.

Many studies confirm the hypothesis that patients harboring identifiable residual leukemic cells during morphologic remission are likely to experience a more rapid leukemic relapse and that the presence of these unusual cells may indicate more aggressive treatment as coupled induction with consolidation chemotherapy or intensification regimens of chemotherapy or rapid bone marrow transplantation after complete remission **[4,16,18,44]**.

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