Flow Cytometry Evaluation of Minimal Residual Disease in Acute Lymphoblastic Leukaemia Type B

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Abstract: Immunophenotyping has become essential to the diagnosis and the treatment management of acute lymphoblastic leukaemia (ALL). We prospectively studied minimal residual disease (MDR) in patients with B lineage ALL who achieved mCR remission. The initial series of patients consisted on 90 cases with B ALL. Sixty-Six patients had bone marrow samples adequate for MDR studies collected on day 35 of remission induction chemotherapy. Strategy of monitoring MRD is based on flow cytometry using quadruple staining according the leukaemia associated immunophenotype found at diagnosis. Data analysis was done using an EPI XL cytometer (Coulter), acquiring 500 000 events. Of the 66 patients 40 (60, 6%) had MRD ≥0, 01%. B lymphoblasts of ALL may morphologically resemble to hematogones (B benign lymphocyte precursors) and their immunophentypes have similarities. Different combinations of antibodies are tested to determine which combinations are more suitable to detect B residual leukaemics cells. The results of this present study indicate that: CD10/CD38/CD19/CD45 and CD10/CD34CD19/CD45 are the more specifics and should be used to distinguish B lymphoblasts of lymphoblastic acute leukaemia from normal hematogones.

Keywords: Acute lymphoblastic leukemia, lymphopoiesis B, flow cytometry, minimal residual disease.

INTRODUCTION

Acute lymphoblastic acute leukaemia (ALL) is a most common leukaemia in childhood with a peak incidence at 2-5 years of age, and another peak in old age (after 50 years). The overall cure rate in children is 85%. Measurement of minimal residual disease (MRD) during clinical remission after chemotherapy has proven to be a valuable tool for predicting relapses before clinical and haematological manifestations and establishing different risk categories in patients with ALL [1]. The detection of residual leukemic cells is usually based on either molecular or immunophenotypical markers present in leukemic but not in normal cells, allowing for their specific discrimination [2]. Studies based on semiquantitative polymerase chain reaction (PCR) or immunophenotyping have demonstrated the clinical relevance of MRD investigation; both methods provide similar results [1, 3]. Evidence supporting the value of MRD investigation in acute leukaemias by immunophenotyping and/or molecular techniques has been increasingly reported over the last few years [1, 2].

Flow cytometry is nowadays the first line method for immunophenotypic identification of blast cells but is not so usual in limited resources countries [4, 5]. In Tunisia, flow cytometry (FC) is used in diagnosis of acute leukaemia and we think that it is the more suitable method to measure MRD than molecular methods which are not done systematically and are more expensive.

Flow cytometry is a practical tool for monitoring MRD in patients with acute lymphoblastic acute leukaemia (ALL). This approach is based on the identification of immunophenotypes expressed by leukemic cells but not by normal lympho-haematopoietic cells in bone marrow and peripheral blood [6]. Flow cytometry measurement of MDR in B ALL present some particularities and difficulties to distinguish in the bone marrow neoplastic lymphoblasts of ALL from benign B-lymphocyte precursors known as hematogones [7, 8].

The aim of this study was analytical: to describe flow cytometric conditions for immunophenotypic analysis at diagnosis of acute leukaemia and to establish a protocol for the measurement of minimal residual disease MRD assayed by four flow cytometry at the end of chemotherapy induction in patients with B ALL in complete morphologic remission using the optimal combination of antibodies. An important question was to determine whether panel of antibodies provided informations of residual cells.

PATIENTS, MATERIALS AND METHODS

Patients

Criteria for entering the study were: Unequivocal diagnosis of B ALL based on morphologic, cytochemical and immunophenotypical criteria [9-11] phenotypically

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aberrant blast cells at diagnosis and morphological complete remission (mCR) with induction therapy.

mCR defined by the criteria proposed by BM VIRDIALES *et al.* [12]:

1. Less than 5% blast cells in regenerated bone marrow (BM).
2. Absence of extramedullary leukaemia.
3. Peripheral blood (PB) Neutrophil count greater than 1.5 10^9/l and platelet count greater than 100 10^9/l.

75 consecutive patients with ALL fulfilled these prerequisites and were included in the present study. The initial series of patients consisted of 201 consecutive patients with *de novo* non acute myeloid leukaemia (non AML). B ALL is diagnosed in 90 cases. Of these patients 85 displayed aberrant immunophenotypes at diagnosis.

Treatment Protocol

Among 85 patients, 75 achieved mCR remission after induction therapy and are the focus of the present study. Distribution according to French- American- British (FAB) classification was/ as follows: L1 (49 patients), L2 (18 patients), L3 (8 patients). All patients were uniformly treated according to the EORTC (European organization for research and treatment of cancer).

The following variables collected at diagnosis were included in the database: age, white blood cell (WBC) count, platelets count, haemoglobin (Hb) level, percentage of blast in BM and absolute number in PB, type of treatment, morphologic FAB classification and karyotype.

BM at Treatment time point: of day 35 are obtained for the prospective investigation of MRD.

Cytological Investigation

Peripheral blood (PB) and Aspiration of bone marrow (sternum puncture in adults and iliac puncture in children) are sent to our laboratory in ethylenediamine tetra-acetic acid tube. After automatic staining of blood and bone marrow smears by using the HEMA TEK slide stainer (AMES Company) and a HEMATEK bloc colorant stain pack (Bayer Diagnostica), cytomorphologic examinations of the blood and the bone marrow slides separately by 2 morphologists are done. Diagnosis of ALL is based on WHO criteria's. Cytochemical analysis is based on pyronine stain showing myeloperoxidase. Cytological exams of PB and BM are done in diagnosis and in post chemotherapy (day 35).

Flow Cytometry Methods

Cell Isolation: Preparation

Cell count specimens were first done on the Coulter LH 750 blood cell counter. The cells were incubated for 15 minutes in the dark with each of the conjugated monoclonal antibodies. Erythrocytes were lysed using lysing solution (optilyse A 11895 - Beckman Coulter) according to the manufacturer's instruction. Following the lysis step, the samples were washed two times with Phosphate buffered saline (PBS). After centrifugation for 5 minutes at 200x g, the supernatant was removed by aspiration. The cell pellet was conserved at + 4 °C. The cells were resuspended in PBS for acquisition.

Antibodies

Antibodies to the following antigens were used in diagnosis and to specially profile B cell precursors in the measurement of MRD in ALL BCoulter- Immunotech):

IgG1 Fluorescein Isothiocyanate (FITC), HLA-DR (FITC), CD34 (FITC), CD7 (FITC), CD3 (FITC), CD10 (FITC), CD22 (FITC), CD15 (FITC), CD65 (FITC), CD3 (FITC), CD29 (FITC), CD3IntraCytoplasmique (IC) (FITC), CD16 (FITC), CD19 (FITC), CD4 (FITC), CD56 (FITC), CD29 (FITC), Lambda (FITC), Kappa (FITC), IgG1 R-Phycoerythrin (PE), CD34 (PE), CD2 (PE), TCR (PE), CD5 (PE), CD20 (PE), CD22 (PE), CD19 (PE), CD38 (PE), CD79a (PE), CD33 (PE), CD13 (PE), CD117 (PE), MPO (PE), CD14 (PE), CD1a (PE), CD8 (PE), CD58 (PE), CD 19 Allophycocyanin (APC), CD45 R- Phycoerythrincyanin 5.1 (PC 5).

IntraPrep Permeabilization Reagent (Beckman coulter) was used for immunological detection of intracellular leukocyte antigens (CD79a, CD3, MPO, Kappa, Lambda) after a formaldehyde/saponin-based permeabilization. The procedure consists of first, fixing cells with Reagent 1 (formaldehyde), and second, permeabilizing the white cells and lysing red cells using Reagent 2 (saponin).

For compensation of the cytometer we used FLow check. (Réf: 6605359) and Cyto-Comp. (Réf: 6607023).

Protocols

First step: Flow cytometry using triple staining for diagnosis:

Erythrocyte-lysed whole PB and BM samples obtained at diagnosis were analysed, with a large panel of 3 colour combinations of monoclonal antibodies according Table **1**, for the identification of phenotypic aberrancies that could be used later in the study of MDR For each experiment, 10 000 cells were measured.

Second step: Immunophentypical investigation of MRD:

Erythrocyte-lysed whole BM samples obtained at 35 day of treatment were analysed by multiparametric flow cytometry using quadruple staining according the leukaemia-associated immunophenotype found at diagnosis. For each experiment, 500 000 cells were measured.

Flow Cytometry Interpretation

PB and BM Samples were acquired with a three and four colour flow cytometer for diagnosis, identification of phenotypic aberrancies and the measurement of MDR. Data analysis was done using an EPICS XL Flow cytometer with Software system II version 3 (Beckman Coulter).

Distinct cell populations (clusters) were identified based on any combination of forward (FSC) and orthogonal light scatter properties (SSC) and fluorescence intensity with various antibody combinations. Each specimen's event clusters were considered positive or negative compared with the degree of the same specimen stained with the isotypic control antibody.

Table 1. Protocol of Flow Cytometry Analysis at Diagnosis

N° of Tubes	*FITC* Fluorescein Isothiocyanate	*PE* R-Phycoerythrin	*PC5* R- Phycoerythrin-Cyanin 5.1
1	IgG1	IgG1	CD45
2	HLA DR	CD34	CD45
3	CD7	CD2	CD45
4	CD3	TCR	CD45
5	CD10	CD5	CD45
6	CD22	CD20	CD45
7	CD10	CD19	CD45
8	-	CD79a (c)	CD45
9	CD34	CD33	CD45
10	CD15	CD13	CD45
11	CD65	CD117	CD45
12	CD3(c)	MPO (c)	CD45
13	CD16	CD14	CD45
14	CD19	CD1a	CD45
15	CD4	CD8	CD45
16	CD56	CD3	CD45
17	CD10	CD38	CD45
18	CD29	CD58	CD45
19	CD34	CD22	CD45
20	HLADR	CD20	CD45
21	Lambda	Kappa	CD45

c: cytoplasmic.

Analysis following gating :

- Selection of mononuclear cells (A) on the histogram Side scatter (SSC) / Forward Scatter (FSC).
- Detection of lymphoblast at diagnosis (B): Gating on histogram SSC/CD45 (SSC low, CD45low).
- Detection of B cell in MDR analysis: Gating (CD19+ CD45 low).

Immunophenotype of the AL was defined following EGIL (European Group for the Immunological Characterization of Leukaemia) recommendations. B lineage ALL is defined by the expression of at least two of the following three early B cell markers: CD19, CD79a and/or CD22 [10].

We prospectively measures the percentage of residual leukaemic cells among bone marrow mononuclear cells collected after remission reinduction (day 35) in the 75 patients with a leukaemia - associated immunophenotype. Statistical analysis was done on logiciel (EPI INFO Version 6).

RESULTS

As mentioned, this study is based on 75 consecutive patients with B lineage ALL diagnosed in cytology as: L1/ L2: 49/18 cases, Burkitt: 8 cases (Table **2**) in whom blast cells displayed antigenic phenotypic aberrancies at diagnosis and who achieved mCR after induction therapy.

Table 2. Clinical Characteristics of the 75 Patients who Achieve Complete Morphologic Remission

	Patient Features
Sex, male/female	39/36 (1,08)
Age, in years	**mean : 18,8 years** **< 2:** 5 (6,6%) **2 to 9:** 30 (40%) **10 to 20:** 18 (24%) **21 to 40:** 12 (16%) **>40:** 10 (13,3%)
WBC count(10^9/L)	2,3 to 320 mean 47
Hb level (g/dL)	3,7 to 13,1 mean: 8,1
Platelets (10^6/L)	1 to 350 mean: 51
Percent blast cells in PB	2 to 95% (60%)
Percent blast cells in BM	20 to 91 % (77%)
FAB classification	**L1**: 49 (65,3%) **L2**: 18(24%) **L3**: 8(10,7%)

Antigenic expression on blast cells at diagnosis was systematically analyzed by multiparametric flow cytometry using triple staining. Following phenotype 69.3% of cases were classified as Pre B (Table **3**).

Table 3. Immunophenotyping Analysis at Diagnosis

B Lineage Acute Lymphoid Leukemia	Subtypes	N	%
CD19+ and/or CD79a+ and/or CD22+	B-I (Pro-B)	1	1,3
	B-II (Common)	14	18,7
	B III (Pre-B)	52	69,3
(75 cases)	B-IV (Mature B)	8	10,7

Table **4** resumes the frequencies of aberrant phenotypes found by flow cytometry. 27 leukaemia had one aberrant marker (39%), 11 (14.7%) leukaemia had more than one aberrant marker (9 had 2 markers, 2 had 3 markers). CD 33 (myeloid marker) is aberrantly coexpressed in 14.5% of cases (Fig. **1**). Table **5** summarizes the leukaemia associated characteristics used to monitor MRD in the B ALL.

Table 4. Frequencies of Aberrant Phenotypes

Aberrant Phenotypes	Number of Cases	%
Cross Lineage Infidelity		
T lympoid cross lineage expression		
CD5	11	14,5
CD7	7	9,5
CD2	2	2,7
CD4	2	2,7
CD8	1	1,3
Myeloid cross lineage expression		
CD33	14	18,7
CD13	6	8
CD117	1	1,3
CD65	2	2,7
NK absurd marker		
CD56	4	5,3
Monocytic absurd marker		
CD14	2	2,7
Antigen over or Underexpression of Markers		
Overexpression		
CD10	55	73,3
CD34	7	9,5
CD19	2	2,7
Underexpression		
CD10	7	9,5
Asynchronous Antigen Expression		
CD10 and CD20	4	5,3
CD22 and CD34	6	8

Immunophenotypic analysis of the BM in mCR demonstrated that based on the level of residual phenotypically aberrant cells displaying a leukaemia - associated phenotype (LAP+): MRD is detected at less than 10^{-4} level (10/10 000cells). The level of MRD is defined by the number of lymphoblasts of ALL /10 000 of all acquired cells (Fig. **2**), 9 of the 75 could not be assessed for MDR measurement for a variety of technical reasons, including lack of adequate numbers of viable cells in the sample or blood dilution (> 50% of neutrophils PNN in BM). Of the 66 patients who achieve mCR and had sufficient cells for flow cytometry studies of MRD, 40 (60.6%) had leukemic cells identifiable by flow cytometry in the BM. Among these patients, the levels of leukaemia were 0.01-<0.1% in 8 patients (12.1%), 0.1-<1% in 20 (30.3%) and ≥ 1 in 12 (18.1%) (Table **6**). Table **7** resumes the level of MRD according clinical and cellular characteristics. The presence or absence of residual leukaemia by flow cytometry at day 35 was not significantly related to age, gender or cellular features.

DISCUSSION

The WHO classification of the acute leukaemias incorporates morphologic, immunophenotypic, genetic and clinical features in an attempt to define entities that are biologically homogenous and that have clinical relevance. The acute leukaemias are classified as lymphoid or myeloid based on the lineage of the blast cells. The acute lymphoid leukaemia (ALL) are subdivided into precursor B cell ALL and precursor T ALL [13]. A systematic immunophenotyping of acute leukaemia is also interesting for the detection of leukaemia associated phenotypes which are the basis of the minimal residual disease analysis by flow cytometry [14]. Eligibility criteria for entering the study were unequivocal diagnosis of *de novo* B ALL based on morphologic, cytochemical and immunophenotypically aberrant blast cells at diagnosis [3,10,13] mCR with induction therapy and corresponding mCR bone marrows samples for immunophenotypical investigation of MRD.

The clinical features of the 75 patients include disorders related to anaemia, neutropenia and thrombocytopenia (Table **2**). PB and BM Samples were acquired with a three and four colour flow cytometer for diagnosis, identification of leukaemia associated phenotypes (LAIP) [15] Patterns of cell markers expressed on leukaemic cells but not on normal bone marrow cells, with a large panel and the measurement of MDR. Bradsyock *et al.* [16] were the first to report the use

Table 5. The Immunophenotypic Combinations Used to Measure Minimal Residual Disease in the Bone Marrow Samples of the 75 Patients

	FITC Fluorescein Isothiocyanate	PE R-Phycoerythrin	APC Allophycocyanin	PC5 R- Phycoerythrin-Cyanin 5.1	%
1	CD10	CD38	CD19	CD45	100
2	CD34	CD22	CD19	CD45	66
3	HLA DR	CD20	CD19	CD45	60
4	CD29	CD58	CD19	CD45	60
5	CD34	CD13	CD19	CD45	8
6	CD34	CD33	CD19	CD45	18,7
7	CD10	CD34	CD19	CD45	100

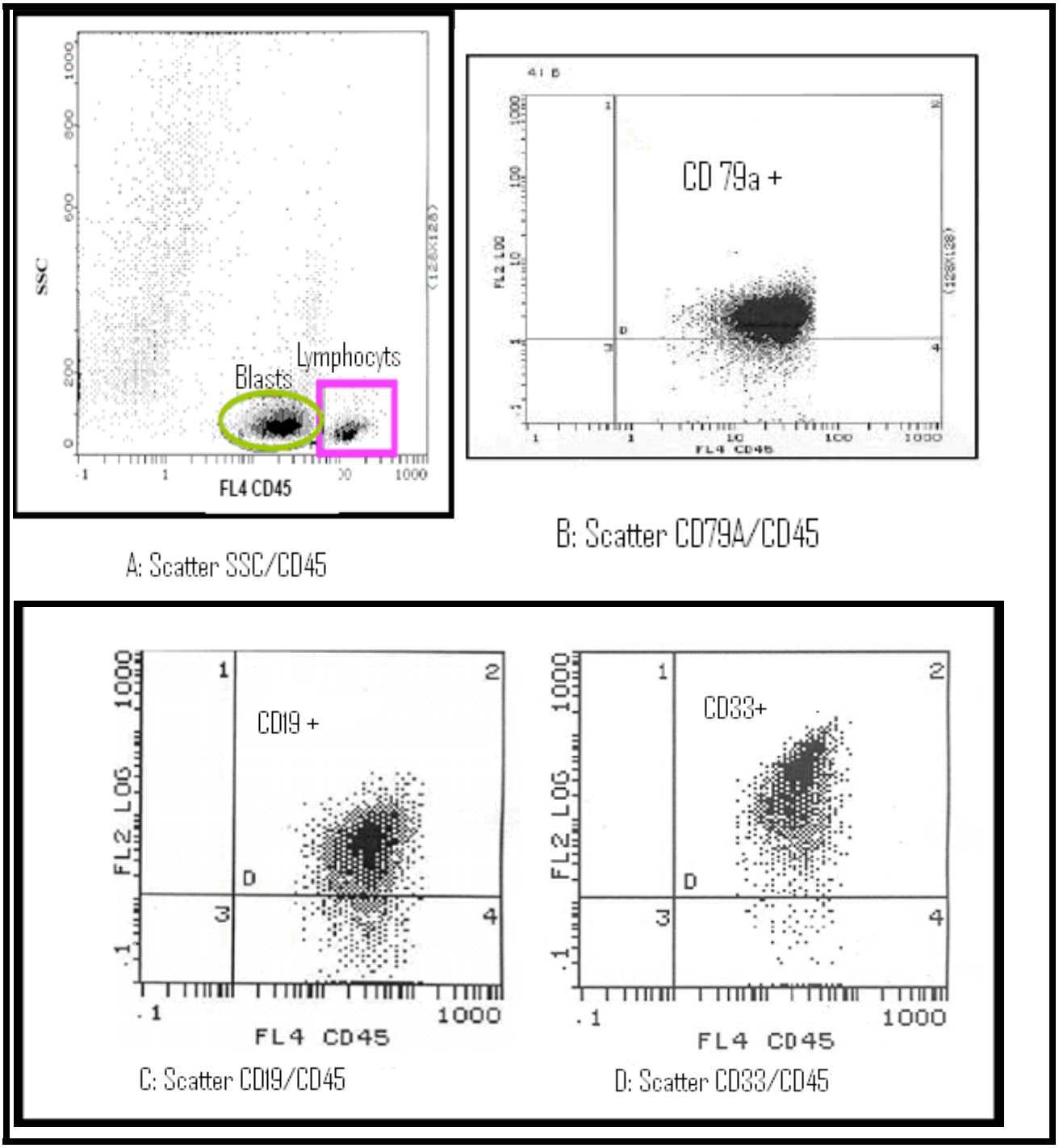

Fig. (1). Immunophenotype of one B ALL case identified one leukaemia associated immunophenotype at diagnosis: Blasts represented 75% of mononuclear cells and were low SSC (scatter **A**), expressed CD79a (scatter **B**) and CD19 (scatter **C**). CD33 was aberrantly expressed in B lymphoblast (scatter **D**).

of LAIP to investigate several suspicious cells. Similar results are in succession published [17-20].

Flow Cytometry Analysis At Diagnosis

In our series, cases were diagnosed by peripheral blood counts, bone marrow cytology, cytochemistry and immunophenotyping. For flow cytometry analysis we have examined the expression of several lineage and maturation linked antigens used in routine immunophenotyping of patients with AL using a 3 colour step panel. Following phenotype 69.3% of cases were classified as Pre B (Table **3**). Regarding all subgroups, we did not find differences with literature [10, 21]. B lineage ALL is defined by the expression of at least two of the following three early B cell markers: CD19, CD79a or CD22. Four categories of B lineage ALL, designated from BI to BIV were established according to the degree of B lymphoid differentiation of the blast cells [10].

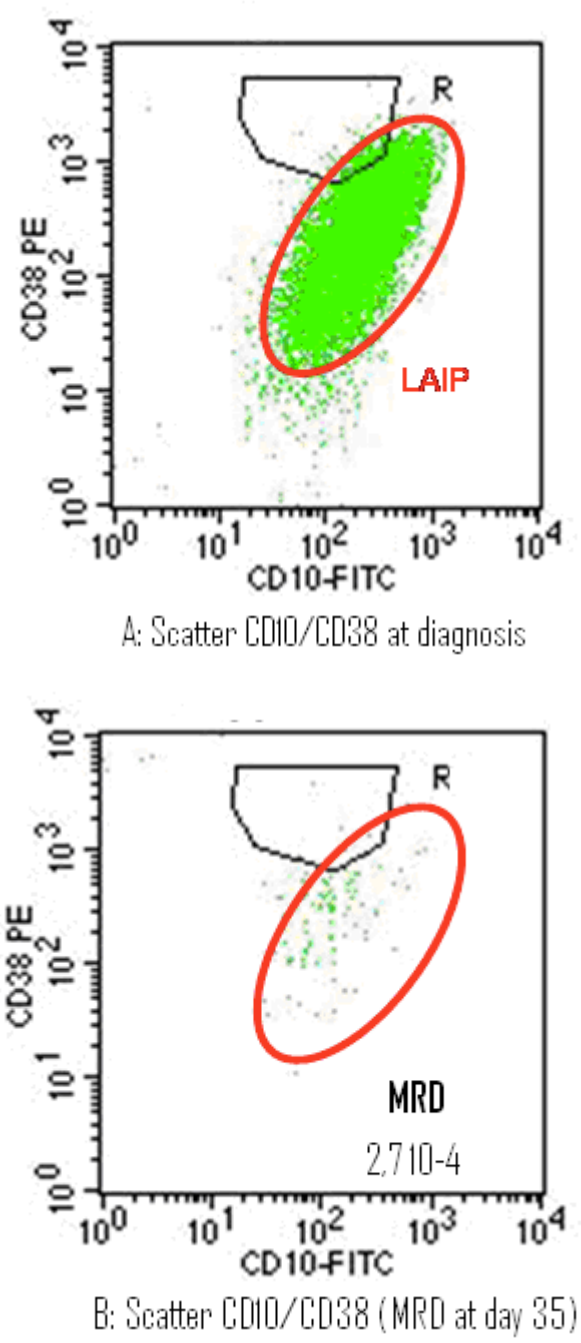

Fig. (2). The detection of minimal residual disease (MRD) on day 35 of morphologic remission induction therapy.

MRD was studied in the 75 patients with B lineage ALL using CD10FITC/CD38PE/CD19APC/CD45PC5. Leukaemia associated immunophenotype(LAIP) was identified at diagnosis (scatter **A**) and it was out of the gating prevue for the normal B cell progenitor (gating R). Residual leukaemia was present at the percentage indicated (scatter **B**).

We used several standardized antibody combinations with a large panel of antibodies to screen ALL samples at diagnosis for leukaemia – associated aberrations. Aberrant characteristics were judged relevant only if they were sufficiently strong or homogenous on a majority of blasts of a leukaemia sample. 4 main types of aberrant phenotypes: Cross-lineage antigen expression (Myeloid/lymphoid), asynchronous antigen expression, antigen overexpression and abnormal light scatter pattern. These phenotypes can identify one leukemic cell among 10 000 normal cells and currently applicable to at least 90% of patients with ALL [3].

Table 6. Minimal Residual Disease at the End of Remission Induction (Day 35) of the 66 Samples with Sufficient Cells for Flow Cytometric Analysis

	<0,01%	0,01-0,1%	0,1-1%	>1%
N (66/75 cases)	26	8	20	12
%	39,4	12,1	30,3	18,2

Table 7. Comparison of Clinical and Cellular Characteristics of Patients who Achieve Low MRD Status (< 0,01% LAP+ Cells) to those who Did Not Achieve this Level (High MRD: ≥ 0,01%) at Day +35

	Cases with MRD Level <0,01% at Day +35	Cases with MRD Level ≥0,01% at Day +35
Age (Years)		
Mean:		
<2	3	1
2-9	15	12
11-20	7	8
>20	8	12
Sex		
Female	16	19
Male	15	16
WBC count (10 9/L)	39	51
Hb level, g/dL	7,9	8,8
Platelets, x 106/L	59	46
Percent blast cells in PB	59	78
Percent blast cells in BM	66	82

Flow cytometry of bone marrow aspirations detected aberrant expression of myeloid or lymphoid T antigens in B ALL. These mixed lineages of leukaemic cells represent the capacity of leukaemia for trilineal expression of leucocytes. For each case, one or more marker combinations that allowed the identification of leukemic cell were selected at diagnosis and applied to study MRD at the end of remission induction. MDR by FC is possible in patients with AL who have aberrant phenotypic features.

Minimal Residual Disease Measurement

BM obtained at the post induction remission were analyzed for detection of MRD by multiparametric flow cytometry using four colour antibodies according to B cell maturation. In order to explore MRD a multivariate analysis must be conducted considering LAIP detected at diagnosis. Detectable MRD was defined as 0.01% or more leukemic cells among mononuclear cells in sample [15].

Our aim was to define within leukemic cells those phenotypes that are frequent, sensitive and specific to

detecting B cell lymphoblasts by using 6 dimensional space formed by the 2 light scatter parameters; forward scatter (FSC) and side scatter (SSC) and the 4 fluorescence-associated characteristics. Several analytical protocols for the study of MRD by FC were described in literature [3,6,21]. A very important limitation for these methods was the identification of patients for MDR study. This is probably because of the technical difficulties related to the immunologic characterisation of acute leukaemias and the detection of LAIP.

According Dario and co-workers, only 35% to 40% are suitable for MRD investigations [17]. Recently, identifying irregularities in the intensity of antigen expression leukaemia by FC define additional LAIP combinations exploitable for MRD. Measurement of MRD by FC is more difficult when the population of interest is rare. We were able to circumvent this problem by acquiring a large number of viable cells (500 000events) and using a gating strategy incorporating different parameters: cell size (FSC), granularity (SSC), expression of CD45 and CD19.

Because the key point of the approach is the identification of phenotypic aberrancies that could be used later for detection of residual blast cells displaying the same phenotypic profile in the BM in mcR obtained after induction therapy: large panels of monoclonal antibodies are needed to cover all the different lymphoid/myeloid lineages and we have designed a gating strategy that can be used to measure residual B cells. However, the neoplastic lymphoblasts of precursor B ALL may morphologically resemble hematogones (benign B lymphocyte precursors), and their immunophenotype also has features in common. Thus, distinction of hematogones and neoplastic lymphoblasts of B cells present in bone marrow may cause diagnostic problems due to their morphologic and immunophenotypic similarities [7, 22-24] Distinction in the bone marrow of benign B-lymphocyte precursors known as hematogones from neoplastic lymphoblasts of ALL is critical for disease management (in post-chemotherapy and post-bone marrow transplant regenerating marrow) [7, 25]. It has been reported that the number of hematogones in bone marrow is variable; the hematogones are present in higher numbers in children and they are often increased in regenerating marrow and in some clinical conditions particularly in patients with cytopenias and neoplastic diseases [7, 26, 27]. Hematogones may be particularly prominent in the regeneration phase following chemotherapy. In some instances they constitute 5 % to more than 50 % of cells [7, 28-30].

The presence of benign immature B cells has been noted to interfere with the flow cytometric analysis of cases of suspected acute lymphoblastic leukaemia because their immunophenotype (positive for CD19, CD10, CD34 and terminal deoxynucleotidyl transferase) is similar to that of pre B cells lymphoblasts and they simulate acute lymphoblastic leukaemia or lymphoma [28, 29, 31]. The presence of hematogones in clinical samples should be recognized so as not to adversely influence prognostic studies [30, 31]. Flow cytometry is reported to distinguish between these cell populations in nearly all instances. In the medical literature that we consulted, the neoplastic lymphoblasts in precursor B ALL deviate from the normal B-lineage maturation spectrum and exhibit maturation arrest and over-, under-, and asynchronous expression of antigens observed on normal B-cell precursors and they often aberrantly express myeloid-associated antigens [22].

Rimsza, has demonstrated that hematogone-rich lymphoid proliferations exhibit a spectrum of B- lymphoid differentiation antigen expression with predominance of intermediate and mature B lineage cells [8]. Flow cytometry revealed in this study that intermediately differentiated cells (CD10+, CD19+) predominated followed in frequency by CD20+ [8]. Hematogone populations always exhibit a continuous and complete maturation spectrum of antigen expression typical of the normal evolution of B-lineage precursors; they lack aberrant or asynchronous antigen expression [22]. Hematogones are precursors which were defined by CD19 positivity and CD45 bright, the expression of antigen of immaturity: HLA DR and CD34, and the coexpression of more mature markers CD19, CD20, CD22. These cells are blended and confused with those of mature B lymphocytes (CD10 negative) on CD45/SSC and could be better recognized on CD10 gating [7]. With flow cytometry using optimal antibodies in combination, the distinction can nearly always be made. However, we have to emphasize the difficulties in distinguishing these cells from residual marrow blasts after chemotherapy [23, 24, 32]. Identification of normal hematogones B contribute to better clarify the detection of small numbers of blasts B of acute lymphoblastic leukaemia [3, 15].

An important question is mentioned in all series describing flow cytometrics measurements of residual disease and using different combinations of antibodies with large panels: which panel of monoclonal antibodies provided more informations of residual cells? The result of this present study indicates that two combinations are suitable for the monitoring of MRD by FC in B ALL: CD10/CD38/ CD19/CD45 and CD10/CD34/CD19/CD45, because they allow the screening of LAIP which are frequent and distinguish B lymphoblasts from normal hematogones. As shown in the FC data analysis, our results are entirely satisfactory and in general, are in concordance with literature [12, 25]. The strategy of monitoring MRD described previously is relatively simple and standardized but remains limited by the quality of FC analysis at diagnosis and screening of aberrant phenotypes.

CONCLUSION

FC is a very powerful approach to study MRD. The result of this prospective study proposes a strategy to monitoring MDR by FC that could be helpful in the treatment stratification and the management of patients with B lineage ALL. A strong correlation between flow cytometric measurements of MRD during clinical remission and treatment outcome has been demonstrated, suggesting that these assays should be incorporated into treatment protocols. For that purpose, new analytical approaches must be performed in order to be used in the investigation of MDR.

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