Myeloid enzymes profile related to the immunophenotypic characteristics of blast cells from patients with acute myeloid leukemia (AML) at diagnosis*

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The purpose of this study was to assess the possible relationship between the cytochemical enzyme profile and immunophenotypic characteristics of distinct acute myeloid leukemia (AML) subtypes in discrete stages of leukemic cells maturation. As the proportion of leukemic blast cells is critical for exact cytochemical analysis, study was restricted to the evaluation of 48 adult and pediatric patients with newly diagnosed AMLs with 80% or more blasts in analyzed samples. The cytochemical investigation of myeloperoxidase (MPO), Sudan black B (SBB), chloroacetate esterase (CAE), α-naphthyl butyrate esterase (ANBE), α-naphthyl acetate esterase (ANAE) and acid phosphatase (AP) in peripheral blood and/or bone marrow was performed. The immunophenotype was examined for the maturation dependent myeloid antigens CD13, CD33, CD11b, CD14, CD15, CD65, CD36, cytoplasmic MPO, non-lineage associated CD34 and HLA-DR antigens, lymphoid-associated antigens CD7, CD4, CD38 as well as natural killer cell associated marker CD56. Flow cytometry by double marker staining and visualization of pathologic cells in dot plots reflected immunophenotypic aberrancy and degree of cell maturation. The patients were classified into AML subtypes M0-M2, M3, M4 and M5 according to the main morphological, cytochemical and immunophenotypical features. The variable combinations of MPO, SBB, CAE and ANBE were identified in relation to immunophenotype. The cytochemical profile of blasts was in concordance with immunophenotype, particularly in more differentiated AML subtypes, M3, M4 and M5. The findings of myeloid antigens expression and cytochemical features in poorly differentiated AML subtypes showed no practical relevance of cytochemical analysis. Notwithstanding that the cytochemical analysis of AML subtypes not sufficiently identifies the distinct aberrancies in heterogeneous leukemic blast cell populations, evaluation of the cytochemical profile in connection with immunophenotyping may help to classify the AML patients to relevant subtypes with more accuracy.

Key words: acute myeloid leukemia, immunophenotype, cytochemistry, myeloid enzymes

AML is a heterogeneous group of leukemias characterized by the blockage of myeloid differentiation at different maturation stages, which define distinct subtypes. Several subpopulations of the leukemic clone can be involved and the stage of the maturation arrest may have an impact on the ability to identify pathologic cells precisely [1, 6, 25].

Although the French-American-British (FAB) morphologic/cytochemical criteria still play a relevant role in the initial AML diagnosis and basic characterization of distinct maturation features [9, 10], immunophenotyping is nowadays an important method [5, 6, 7, 13]. In fact, imunophenotyping by the flow cytometry has ability to precisely identify, characterize and enumerate the myeloid blast cells. By immunophenotyping it is possible to discriminate the pathologic cells from normal cells, identify the aberrant phenotypes of blast cells, to define the cross-lineage antigen expression, antigen overexpression, asynchronous antigen expression, absence of lineage specific antigens and abnormal FSC/SSC position of leukemic blasts visualized in the dot plot [3, 5, 6].

The precise pathologic classification of blast cells, besides the cytomorphology, cytochemistry and immunophenotyping requires in some cases also the detection of cytogenetic abnormalities for prognostic and therapeutic purposes [2, 4, 8].

Leukemic myeloblasts express a variety of leukocyte differentiation antigens, which reflect commitment to the myeloid lineage, level of maturation as well as provide the information on the degree of blast cells heterogeneity [6, 7, 13, 25]. AML constitutes the group of leukemias composed by

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less (M0, M1, M5a), more (M2, M3, M5) differentiated and by single (M1, M3, M5) or mixed (M4, M6, M7) cell lineage leukemic blasts [6].

On the other hand, the presence of cytochemically demonstrated myeloid enzymes, MPO, SBB, non-specific and specific esterases (ANAE, ANBE, CAE) in the blast cells represent another important marker of myeloid origin of AML with possibility to characterize its maturation degree [15, 17, 20].

Literature data comparing flow cytometry and enzyme cytochemistry are limited [14, 20, 23].

The EGIL (The European Group for Immunological Classification of Acute Leukemias) reported following: "it is agreed by the group that all the data must be referred to the blast cells" [7]. According of this statement it is supposed that the exact cytochemical identification of AML subtypes can be conditioned by the percentage of blasts in analyzed samples.

The purpose of the present study was to evaluate critically the association between the cytochemical enzyme profiles and immunophenotypic features of blast cells in the context of the discrete stages of leukemia cells maturation in the AML patients, whose bone marrow and/or peripheral blood were infiltrated with 80% or more leukemic blast cells at diagnosis. In addition, we looked for the possible discrepancies between the cytochemical and immunophenotypic results. Finally, we judged the role of enzyme cytochemistry in classification of AML subtypes.

Material and methods

Patients and leukemic samples. A total of ninety-one patients, both children and adults, with the newly diagnosed, previously untreated acute myeloid leukemias (AMLs) were included in this study. All patients had originally been evaluated in the period of December 1999 to May 2004, according to the criteria of the FAB cooperation group on the basis of May-Grünwald-Giemsa staining smears of peripheral blood (PB) and/or bone marrow (BM), integrated by the enzyme cytochemical analysis and immunophenotyping. As the number of pathologic cells is considered being critical for the precise cytochemical diagnosis of AML subtypes, we selected from these cases those with 80% or more leukemic blast cells in analysed samples. The selected group with 80–100% blast cells in PB and /or BM consisted of 48 patients. The median percentage of blast cells was 89.3±1.8. There were 24 males (50%) and 24 females (50%). Six of the patients (12.5%) were children with a median age of 8.1±5.1 years (range 1–15). The median age of 42 adult cases (87.5%) was 56.8±17.2 years (range 21–82). The mean WBC count for all patients was 96.7±94.8.

In terms of FAB criteria, 30 of 48 selected cases (62.5%) belong to less mature M0/M1/M2 AML subtypes (3 were AML-M0, 17 M1, and 10 M2), ten of patients suffered from AML-M3 (16.7%). Five cases were AML-M4 (10.4%) and five were AML-M5 (10.4%). The AML subtypes M6 and M7 were not observed among the followed patients. The controls were 10 subjects who underwent BM aspiration for clinical diagnosis but whole BM and PB contained no pathologic cells.

Enzyme cytochemistry. The AMLs were diagnosed with help of light microscopy cytochemistry. PB and BM cells were routinely stained for the presence of myeloperoxidase (MPO), Sudan black B (SBB), naphthol AS-D chloroacetate esterase (CAE), α-naphthyl acetate esterase (ANAE) inhibited or not by sodium fluoride, α-naphthyl butyrate esterase (ANBE) and acid phosphatase (AP) (Sigma-Aldrich Chemie GmbH, Germany) by a standard procedures. At least 300 cells were counted to determine the percentage of positive cells. Stain positivity for MPO and SBB was determined if the reactivity was present in 5–10% of the blast cell population but CAE and ANBE required >20% positivity.

Immunological marker analysis. Fresh, heparin anticoagulated PB and/or BM samples were immunophenotyped at diagnosis. Analysis of leukemic cells was performed either after the isolation by a standard gradient technique or by using an erythrocyte lysed-whole blood method with Optilyse as a lysing agent (Optilyse B, Immunotech, France). The cell surface and cytoplasmic antigens were detected using single and/or dual color techniques with fluorescein isothiocyanate (FITC) and phycoerythrin (PE) conjugated monoclonal antibodies (MoAbs). A large panel of MoAbs defining the myeloid lineage (CD13, CD33, anti-MPO), degree of myeloid cell differentiation (CD15, CD11b, CD14, CD65, CD36) and cell immaturity/non lineage restricted (CD34, HLA-DR, CD45) was utilized. The CD4, CD7 and CD38 as lymphoid cell markers and the natural killer (NK) cell associated marker CD56 were also used. MoAbs were purchased from Immunotech, Beckman Coulter Company, Marseille, France. Flow cytometric analysis was performed on both FACStar (Becton-Dickinson, USA), where data were analyzed using Consort 30 Data Management System and EPICS ALTRA flow cytometer (Beckman Coulter International S.A, USA) equipped by Expo 32 program for analysis.

In some cases with negative MPO cytochemistry, the cytoplasmic detection of MPO using an anti-MPO (clone CLB-MPO-1, Immunotech, France) in fixation/permeabilization IntraPrep method according to the instructions proposed by Immunotech (Beckman Coulter, Marseille, France) was performed.

Identification of leukemic blast cells was performed using forward (FSC) versus side scatter (SSC) parameters and/or CD45 intensity versus SSC dot plots. Gating of cells was important to differentiate between pathologic blasts and normal cells. The results of each antigen were expressed as percentage positivity stained cells within the gated blasts population. In all cases, isotype-matched immunoglobulins with no reactivity to PB and BM cells were used as negative control.

Statistical analysis. Student's t-test for equal and unequal variance was used to analyse the statistical significance of the results. P values <0.05 were considered significant.

Results

The main characteristics of AML patients, both children and adults, evaluated in this study at diagnosis are summarized in Table 1. Forty-eight (48) selected cases with 80% or more blast cells were included in the study. The mean percentage of blasts in analyzed PB and BM samples was 89.3±1.8 (range, 80–100). The median WBC count was 96.7±94.8 and did not differ significantly among the individual AML subtypes for the wide range of data variation (6–415 x 10^9/l).

Table l. Characteristics of AML patients at diagnosis

Number of patients	48
Sex	
Male	24 (50%)[a]
Female	24 (50%)
Age (years)	
Adults (42 patients, 87.5%)	56.8 ± 17.2 (21–82)[b]
Children (6 patients, 12.5%)	8.16 ± 5.1 (1–15 years)
WBC (x10^9/L)	96.7 ± 94.8 (6–415)[b]
Percentage of LBC (range)	89.3 ± 1.8 (80–100)
FAB	
M0/M1/M2	30 (62.5)[a]
M3	8 (16.7)
M4	5 (10.4)
M5	5 (10.4)

[a]number of patients (%), [b]median ± SD (range), LBC – leukemic blast cells

The AML patients were diagnosed by morphology and cytochemistry according to the FAB criteria and by immunophenotyping. Cases were categorized according to the degree of maturation into AML subtypes as follows: AML-M0/M1/M2 (total 30 patients, 62.5%; M0 in 3 cases, 6.3%; M1 in 17, 35.4% and M2 in 10 patients, 20.8%), AML-M3 in 8 patients (16.7%), AML-M4 in 5 cases (10.4%) and AML-M5 (10.4%). Table 2 shows the antigens distribution in AML patients. The cytochemical profile of AML patients is recorded in Table 3.

In the present study we looked for the possible relation between the immunophenotypic characteristics and cytochemical enzyme profiles in distinct subtypes of AML patients.

It appears that three of AML patients fulfilled the criteria of M0 subtype. The high expression of cytoplasmic MPO, negativity for CD13 and CD33 antigens, the CD34, HLA-DR and CD7 co-expression, lack of expression of lymphoid-specific antigens, along with more than 90% of blast cells (91.3±2.3) in gated population were the characteristic immunophenotypic features. Moreover, cytochemical staining for the myeloid enzymes MPO and SBB was negative (less than 3% of cytochemically detected MPO or SBB positivity) (Tab. 2, 3, 4; Fig. 1). Some of blast cells showed a faint diffuse positivity for AP and ANAE (not inhibited by sodium fluoride) (data not shown). Sodium fluoride (NaF) inhibits the enzymatic activity in monocytes.

The differences between AML-M1 (without maturation) and M2 (with maturation) subtypes in their immunophenotypic characteristics are shown in Table 2. The percentage of blast cells was relatively similar (87.8±5.4 and 90.8±4.2, respectively) (Tab. 4). The varying number of patients expressing CD13 and CD33 in their blast cells was found. The CD34 expression was more frequent in AML-M1 than that in M2 patients. HLA-DR was found in the majority of patients. Co-expression of CD7 and CD38 with myeloid markers was seen to decrease in AML-M2 patients. The number of patients with maturation dependent CD11b and CD65 antigens expression was higher in AML-M2 than those in AML-M1 ones. One of eight AML-M2 patients displayed monocytic CD14 antigen expression.

Cytochemically detected MPO and SBB were the characteristic features of blast cells of all AML-M1 and M2 patients. The CAE (granulocyte differentiation marker) appeared in blast cells of some patients with AML-M2 subtype. Myeloid blasts lacked the positivity for ANBE in both M1 and M2 subtypes (Tab. 2, 3, 4; Fig. 2, 3, 7, 8, 9). On the other hand, blast cells of some patients with AML-M1 subtype preserved its AP and ANAE positivity (no inhibited by sodium fluoride) (data not shown).

The immunophenotype of AML-M3 subtype, with the range of promyelocytic blast cells from 84 to 95% (91.0±4.7), was characteristic by the expression of CD33 and CD13, CD11b and CD65 antigen. The CD56 expression was observed in 25% of patients. The CD14 expression was not noticed. The absence of CD34 and HLA-DR was the most characteristic feature of this subtype (Tab. 2).

Cytochemical evaluation demonstrated strongly positive reaction for MPO (87.0±8.1% positive cells in the blast population) and SBB (91.8±5.5%) in all patients, CAE positivity (range from 24 to 64%) was seen in 5 of 8 cases. No subset of blast cells showed positivity for ANBE (Tab. 3, 4; Fig. 4, 7–10).

The immunophenotype of acute myelomonocytic leukemia (AML-M4) with the median percentage of blast cells 88.4±5.9 (range 80–95%) represented an immunologically heterogeneous population with more mature cells predominance. The expression of CD33, CD11b, CD65 and HLA-DR was found in all of patients. Many of patients expressed CD13, CD14, CD15 and CD4 antigen (Tab. 2).

By cytochemical analysis, marked enzymatic heterogeneity was noticed, with a variable proportion of positive blast cells. All patients displayed positivity for MPO (16.5±13.6% of positive blasts, range 10–43), SBB (45.2±29.5%, range 14–89), CAE (40.2±6.7%, range 34–50) and ANBE (54.5±15.4%, range 34–59) (Tab. 3, 4, Fig. 5, 7–10). The proportion of pathologic cells with the features of both,

Table 2. Immunophenotypic characteristics of patients in AML subtypes

Antigen	M0 n=3	M1 n=17	M2 n=10	M3 n=8	M4 n=5	M5 n=5
HLA-DR	3/3 (100)[a]	16/17(94)	8/10 (80)	0/8 (0)	5/5 (100)	5/5 (100)
CD34	3/3 (100)	9/16 (56)	3/9 (33)	0/8 (0)	2/5 (40)	0/5 (0)
CD13	0/3 (0)	10/17 (59)	8/10 (80)	2/8 (25)	2/5 (40)	1/5 (20)
CD33	0/3 (0)	12/17(71)	9/10 (90)	8/8 100)	5/5 (100)	5/5 (100)
cMPO	3/3 (100)	6/6 (100)	ND	ND	ND	ND
CD11b	0/3 (0)	4/12 (33)	5/9 (56)	4/8 (50)	5/5 (100)	5/5 (100)
CD14	0/3 (0)	0/16 (0)	1/8 (12)	0/8 (0)	3/5 (60)	4/5 (80)
CD15	0/3 (0)	0/7 (0)	0/4 (0)	ND	2/5 (40)	2/3 (67)
CD65	0/3 (0)	1/15 (7)	2/8(25)	3/8 (37)	5/5(100)	2/5 (40)
CD7	3/3 (100)	9/13 (69)	1/5 (20)	0/8 (0)	0/5 (0)	0/5 (0)
CD38	0/2 (0)	10/15(67)	3/6 (50)	0/4 (0)	1/5 (20)	1/5 (20)
CD4	ND	ND	ND	ND	2/5 (40)	3/5 (60)
CD36	ND	ND	ND	ND	0/5 (0)	1/5 (20)
CD56	ND	0/6 (0)	0/2 (0)	2/8 (25)	0/5 (0)	2/5 (40)

[a] number of antigen positive patients/number of tested patients (% of positive cases)

Table 3. Cytochemical features of patients in AML subtypes

Enzyme	M0 n=3	M1 n=17	M2 n=10	M3 n=8	M4 n=5	M5 n=5
MPO	0/3 (0)[a]	17/17(100)	10/10(100)	8/8 (100)	5/5 (100)	5/5 (100)
SBB	0/3 (0)	17/17(100)	10/10(100)	8/8 (100)	5/5 (100)	5/5 (100)
CAE	0/3 (0)	0 /15 (7)	4/10 (60)	5/8 (63)	3/5 60)	0/5 (0)
ANBE	0/3 (0)	0/17(0)	0/10 (0)	0/8 (0)	5/5 (100)	5/5 (100)
AP	3/3 (100)	3/11 (27)	0/6 (0)	ND	ND	ND
ANAE*	3/3 (100)	3/11 (27)	0/6 (0)	ND	ND	ND
ANAE**	ND	ND	0/6 (0)	ND	5/5 (100)	5/5 (100)

[a]number of enzyme positive patients/number of tested patients (% of positive cases), ND – not done, *ANAE not inhibited by sodium fluoride, **ANAE inhibited by sodium fluoride

Table 4. Relationship between the percentage of positivity of myeloid enzymes and AML subtypes

	M0 n=3	M1 n=17	M2 n=10	M3 n=8	M4 n=5	M5 n=5
% of blasts	91.3±2.3[a] 90–94 [b]	87.8± 5.4 80–97	90.8± 4.2 84–100	91.0± 4.7 84–95	88.4± 5.9 80–95	87.0±4.7 81–93
MPO	< 3%	36.7±30.7[c] 11–84[b]	58.2±19.8 27–97	87.0± 8.1 78–97	16.5±13.6 10–43	9.7±8.9 7–25
SBB	< 3%	58.1±32.3 10–97	80.5±16.8 43–97	91.8± 5.5 82–98	45.2±29.5 14–89	10.6±6.1 7–21
CAE	neg	neg	37.0±14.2 24–61	41.1±20.9 24–64	40.2± 6.7 34–50	neg
ANBE	neg	neg	neg	neg	54.5±15.4 34–59	86.4±5.0 80–92

[a]median percentage of blast cells ± SD, [b]range, [c]mean percentage of enzyme positive cells ± SD

granulocyte and monocyte in the single cell displayed a combined staining of blasts with CAE and ANBE. Three types of cells were found, myeloid cells with only CAE, monocytes with only ANBE and cells with myelomonocytic appearance (CAE and ANBE in the same blast) (data not shown).

The immunophenotype of acute monocytic leukemia (AML-M5) patients with the 87.0±4.7% (range 81–93) of blast cells in gated population was associated with the expression of HLA-DR, CD33, CD11b, CD15, CD65, CD4, CD36 and CD56. The CD14 antigen, whose expression is considered as a marker of monocytes, was positive in four of five patients (80%) (Tab. 2).

Cytochemical profile of AML-M5 subtype exhibited the high proportion of blasts positive for ANBE (86.4±5.0%, range 80–92) in all tested patients. We consider this monocyte marker to be more sensitive and selective than that of ANAE inhibited with sodium fluoride. According to our experience, the monocyte staining reaction with α-naphthyl butyrate as substrate appeared too more expressive than that of α-naphthyl acetate. Although the MPO and SBB were found in all patients, the proportion of positive blast cell in population was relatively low (9.7±8.9% and 10.6±6.1%, respectively). The CAE negativity was found in all M5 patients (Tab. 3, 4; Fig. 6–10).

Of note was the discrepancy between the two monocyte markers, ANBE and CD14 in one of AML-M5 patients. While CD14 expression was absent, ANBE was noticed strongly positive in majority of blasts (data not shown).

Cytochemical analysis showed the differences between the distinct AML subtypes in the percentage of positive blast cells for evaluated myeloid enzymes. Statistically significant differences are recorded in Figures 7 (for MPO), 8 (for SBB), and 10 (for ANBE). The statistically significant differences among AML subtypes for CAE positivity were not found (Fig. 9).

There were no remarkable differences between the leukemic blast cells of PB and BM and between pediatric and adults in the main immunological and cytochemical

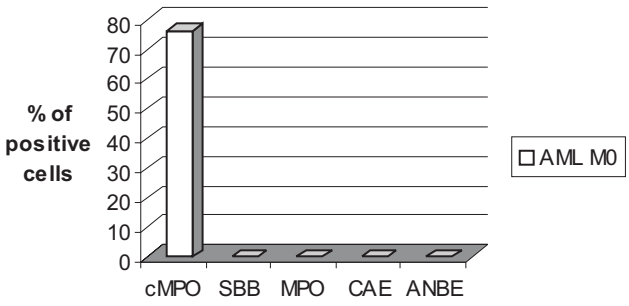

Figure 1. The percentage of cytoplasmic myeloperoxidase (cMPO) detected by immunophenotyping with anti-MPO antibody in AML-M0 subtype (n=3).

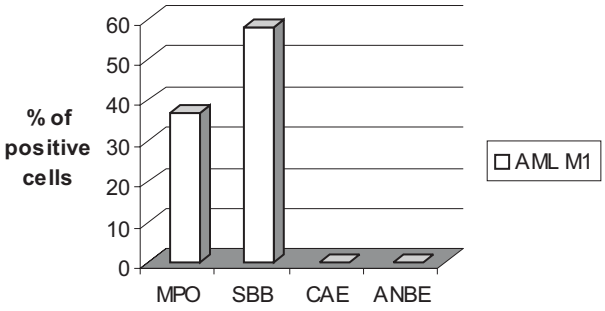

Figure 2. Cytochemical characteristics of AML-M1 subtype. MPO, SBB (n=17).

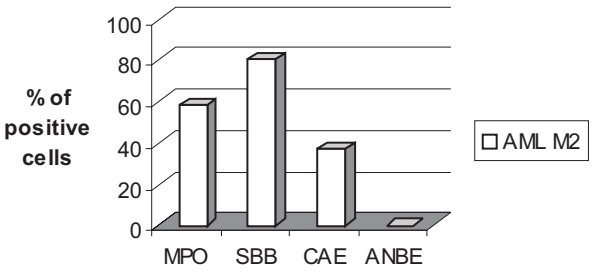

Figure 3. Cytochemical features of AML-M2 subtype. MPO, SBB (n=10), CAE (n=4).

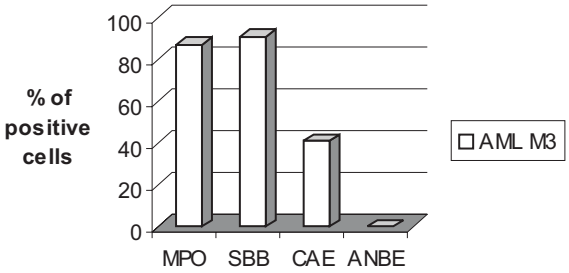

Figure 4. Cytochemical characteristics of AML-M3 subtype. MPO, SBB, CAE (n=8).

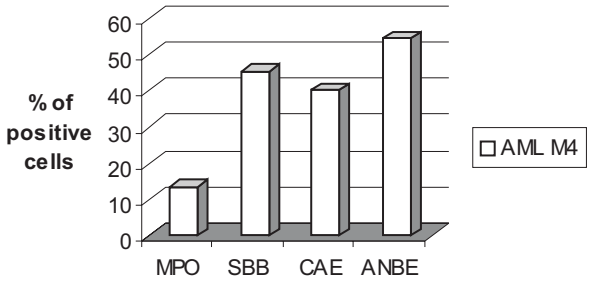

Figure 5. Cytochemical profile of AML-M4. MPO, SBB (n=5), CAE (n=3).

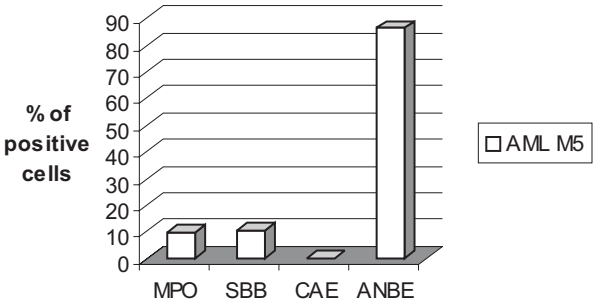

Figure 6. Cytochemical profile of AML-M5 subtype. MPO, SBB, ANBE (n=5).

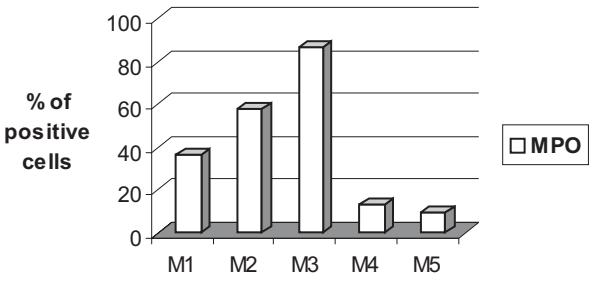

Figure 7. The percentage of MPO positive blasts in distinct AML subtypes. Statistically significant were the differences between subtypes: M1–M3, p=0.0004; M1–M5, p=0.0168; M2–M3, p=0.0075; M2–M4, p=0.0003; M3–M5, p=0.0001.

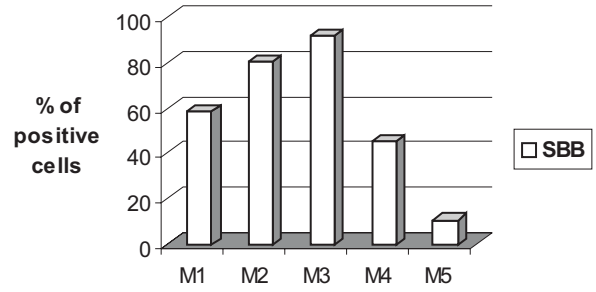

Figure 8. The percentage of SBB positive blasts in AML subtypes. Significant differences were between subtypes: M1–M2, p=0.0365; M1–M3, p=0.0009; M1–M5, p=0.0007; M2–M4, p=0.0101; M2–M5, p=0.0005; M3–M4, p=0.0001.

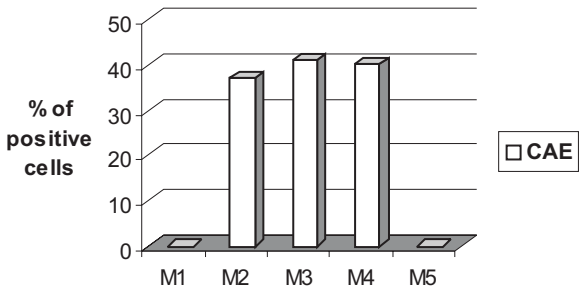

Figure 9. The percentage of CAE positive blast cells in different AML subtypes. No significant differences in CAE positivity in blasts of AML subtypes were observed.

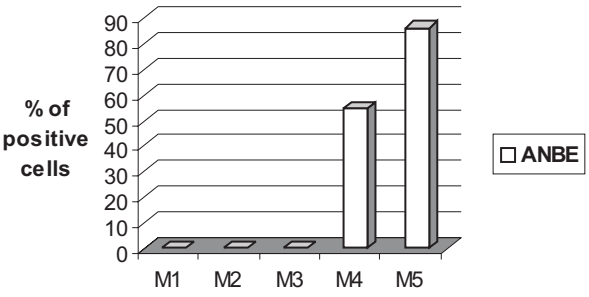

Figure 10. The percentage of ANBE positive blasts in distinct AML subtypes. Statistically significant was the difference between M4–M5, p=0.0058.

characteristics. The children patients in the AML-M3 and AML-M4 subtypes were not observed.

Discussion

The discrimination of pathologic blast cells from normal cells, an accurate identification and determination of their maturation degree as well as the blasts enumeration are the assumptions of the precise AML diagnosis [5, 6, 13]. The morphologic characteristics of the blast cells in the BM and/or PB are essential for the AML differential diagnosis and prognosis [6, 7].

Although the FAB morphologic/cytochemical classification system is the principle for the diagnosis of AML [9, 10], in many cases is not efficient to identify the distinct aberrancies in extremely heterogeneous blast cell populations [1, 5, 6]. In spite of this disadvantage, we suppose that the heavily infiltrated BM and or/PB with leukemic cells may allow the comparison with more accurate immunophenotypic blast cells identification.

Our study was restricted to the evaluation of AML patients with 80 % or more blast cells in analyzed samples at diagnosis to acquire exact cytochemical profile of blasts.

The discrepancies between the cytochemical and immunophenotypic features of blast cells were finding in a small group of patients. The only myeloid associated marker was the positivity for cytoplasmic MPO. Immunologically detected cytoplasmic MPO is the myeloid specific marker because of its early and exclusive expression in the myeloid lineage [8, 16]. The expression of HLA-DR, CD34 and CD7 antigen co-expression confirmed the immaturity of these blast cells [8, 28]. This immunophenotype appeared to fulfill the criteria of very immature AML-M0 subtype [8, 24, 28]. Moreover, the cytochemically detected myeloid enzymes MPO and SBB were absolutely negative [1]. Concerning the diffuse positivity for AP and ANAE not inhibited with sodium fluoride, our results supported the observations of BENNETT et al [10] and VENDITTI et al [28] who described the presence of these enzymes in a portion of blasts in some cases of AML-M0. The cytochemical analysis in this type of AML had no practical relevance in the AML diagnosis.

The revised criteria of FAB classification [9] stated that AML-M1 subtype contains a small subset of relatively maturing (less than 10 % of blasts) cells. The immunophenotypic profile of our AML-M1 patients suggests this finding. Leukemic blasts in some cases, except of CD13, CD33, cMPO, HLA-DR, CD34 expression and co-expression of CD7 and CD38, displayed positivity for CD11b and CD65 antigens, markers of granulocytic stage of maturation. Cytochemically, the AML-M1 blasts exhibited positivity for MPO and SBB that usually parallel each other. However, some cases can deviate from this pattern. The percentage of SBB positive blasts was higher than that of MPO. Occasionally, MPO low positive cases may be associated with stronger SBB positivity. According to CUNEO et al [11] SBB cytochemical staining appeared to be more sensitive than MPO in their study, as the 7 patients, who would have otherwise been classified as AML-M0 because of the presence of less than 3% MPO positive blasts were included among AML-M1 at cytologic review showing 3 % to 12% SBB positive blast cells.

The immunophenotype of AML with granulocytic differentiation, AML-M2 subtype display the positivity for CD13, CD33, HLA-DR and CD34. In addition, more of the patients expressed CD11b and CD65 antigens as it was observed in AML-M1 group [1, 13]. On the contrary, only a few patients co-expressed CD7 and CD38 antigens. Lineage committed myeloid progenitor cells usually expressed very high level of CD38, which decreased dramatically as maturation progresses [26]. Cytochemically, the increased percentage of MPO and SBB positive blasts in AML-M2 as a sign of continuing maturation was observed [27]. Moreover, some blasts displayed CAE positivity, marker of granulocytic differentiation. The usefulness of cytochemistry in the cases of AML M1 and M2 is partial.

Acute promyelocytic leukemia (APL), AML-M3 subtype is considered to be the most differentiated form of AML with the proliferation of malignant, nearly homogeneous popula-

tion of promyelocytes [17, 18, 22]. Characteristic membrane surface feature was the lack of CD34 and HLA-DR [1, 18, 22]. Cytochemically the majority of leukemic blasts revealed very strong MPO and SBB positivity and a variable proportion of CAE, in accordance with the observation of others [1, 17, 18]. Promyelocytic morphology, strong MPO and SBB, loss of HLA-DR and CD34 antigen may effectively exclude the diagnosis of APL from other AML [2]. However, lack of both CD34 and HLA-DR does not always suggest a diagnosis of APL [13, 29]. It seems that in spite of the conclusive promyelocytic morphology and cytochemistry of leukemic blasts in AML-M3 subtype, the full characterization depends on the cytogenetic analysis of t(15;17) translocation [1, 2, 18].

The cytochemical profile of leukemic blasts in acute myelomonocytic leukemia M4 showed the positivity for MPO, SBB, CAE as well as ANBE in a various proportion. The CAE enzyme reaction is usually similar to those with both MPO and SBB, but appears later in myeloid cell development [27]. The discriminative ability of CAE and ANBE was stressed in our cases by both enzymes in combination [15]. Immunophenotype showed the expression in all myeloid lineages-associated and maturation dependent antigens in concordance with others [6,13]. The cytochemical detection of myeloid enzymes appeared to be sufficient to the initial identification of AML-M4 subtype.

The prevalence of the leukemic cells strongly positive for ANBE along with expression of CD14, well characterized our group of monocytic leukemia (AML-M5). The enzyme ANAE (inhibited with sodium fluoride) is considered to be the most cytochemically specific marker of monocyte differentiation [12]. On the other hand, CD14 antigen is an immunologically preferred monocytic marker [30] with prognostic importance [21]. As regards the immunophenotypic characteristics, AML-M5 subtype very often expressed other monocytic markers CD4, CD36, HLA-DR, CD11b and CD56.

The discrepancy in the proportion of leukemic monocytes detected by imunophenotyping (CD14) and by cytochemistry (ANBE) in one patient was noticed. In fact, CD14 antigen is not absolutely restricted to all maturation stages of monocytes. Monoblasts and promonocytes are often CD14 negative [30]. The immature monocytic cells in comparative FERGEDAL's et al [12] study showed no presence of CD14 but ANAE positivity, what was in agreement with our results with ANBE. The high percentage ANBE positive blasts allow the exact cytochemical classification of AML-M5 subtype.

It is supposed, that the cytomorphology and cytochemistry have no relevance for prognosis. However, recently MATSUO et al [19] reported the value of the percentage of MPO-positive blast cells (50%) as a simple and highly significant prognostic factor for AML patients.

In summary, the cytochemical detection of maturation dependent myeloid enzymes is still a useful method that enables to confirm the myeloid or monocytic differentiation pathway of blast cells in AML patients and, when used in combination with immunophenotyping it may help to classify the AML patients to distinct subtypes with more precision. The accurate AML diagnosis may constitute the valuable tool for clinicians to choose an optimal therapy and to provide prognostic information.

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References

[1] ARBER DA. Realistic pathologic classification of acute myeloid leukemias. Am J Clin Pathol 2001; 115: 552–560.

[2] ARBER DA, CARTER NH, IKLE D, SLOVAK ML. Value of combined morphologic, cytochemical and immunophenotypic features in predicting recurrent cytogenetic abnormalities in acute myeloid leukemia. Hum Pathol 2003; 34: 479–483.

[3] BABUŠÍKOVÁ O, KONÍKOVÁ E, KUSENDA J, KOUBEK K. Aberrant markers expression in T- and B-lymphoid and myeloid leukemia cells of different differentiation stages. Neoplasma 1999; 46: 277–282.

[4] BABUŠÍKOVÁ O, SEJNOVÁ D, KIRSCHNEROVÁ G, KIRŠNEROVÁ Z, ČÁP J. Correlation of clinical picture (event free survival and overall survival) in childhood acute leukemia patients with immunophenotype and chromosomal abnormalities. Neoplasma 2000; 47: 382–389.

[5] BAHIA DMM, YAMAMOTO M, CHAUFFAILLE MLF, KIMURA EYS, BORDIN JO, FILGUEIRAS MAC. Aberrant phenotypes in acute myeloid leukemia: a high frequency and clinical significance. Haematologica 2001; 86: 801–806.

[6] BASSO G, BULDINI B, DE ZEN L, ORFAO A. New methodologic approaches for immunophenotyping acute leukemias. Haematologica 2001; 86: 675–692.

[7] BÉNÉ MC, CASTOLDI G, KNAPP W, LUDWIG WD, MATUTES E et al. Proposals for the immunological classification of the acute leukemias. European Group for the Immunological Characterization of Leukemias (EGIL). Leukemia 1995; 9: 1783–1786.

[8] BÉNÉ MC, BERNIER M, CASASNOVAS RO, CASTOLDI G, DOEKHARAN D et al. Acute myeloid leukaemia M0: haematological, immunophenotypic and cytogenetic characteristics and their prognostic significance: an analysis in 241 patients. Br J Haematol 2001; 113: 737–745.

[9] BENNETT JM, CATOVSKY D, DANIEL MT, FLANDRIN G, GALTON DA et al. Proposed revised criteria for the classification of acute myeloid leukemia. A report of the French-American-British Cooperative Group. Ann Intern Med 1985; 103: 620–625.

[10] BENNETT JM, CATOVSKY D, DANIEL MT, FLANDRIN G, GALTON DA et al. Proposal for the recognition of minimally differentiated acute myeloid leukemia (AML-M0). Br J Haematol 1991; 78: 325–329.

[11] CUNEO A, FERRANT A, MICHAUX JL, BOOGAERTS M, DEMUYNCK H et al. Cytogenetic profile of minimally differentiated (FAB M0) acute myeloid leukemia: Correlation with clinicobiologic findings. Blood 1995; 85: 3688–3694.

[12] FERGEDAL M, ÅSTRÖM M, TIDEFELT U, KARLSSON MG. Differences in CD14 and α-naphthyl acetate esterase positivity and relation to prognosis in AML. Leuk Res 1998; 22: 25–30.

[13] KALEEM Z, CRAWFORD E, PATHAN MH, JASPER L, COVINSKY MA et al. Flow cytometric analysis of acute leukemias. Diagnostic utility and critical analysis of data. Arch Pathol Lab Med 2003; 127: 42–48.

[14] KHEIRI SA, MACKERRELL T, BONAGURA VR, FUCHS A, BILLETT HH. Flow cytometry with or without cytochemistry for the diagnosis of acute leukemias? Cytometry (Communications in clinical cytometry) 1998; 34: 82–86.

[15] KLOBUŠICKÁ M. Reliability and limitations of cytochemistry in diagnosis of acute myeloid leukemia. Neoplasma 2000; 47: 329–334.

[16] KONÍKOVÁ E, GLASOVÁ M, KUSENDA J, BABUŠÍKOVÁ O. Intracellular markers in acute myeloid leukemia diagnosis. Neoplasma 1998; 45: 282–291.

[17] LISO V, BENNETT J. Morphological and cytochemical characteristics of leukaemic promyelocytes. Best Pract Res Clin Haematol 2003; 16: 349–355.

[18] LOCK RJ, VIRGO PF, KITCHEN C, EVELY RS. Rapid diagnosis and characterization of acute promyelocytic leukaemia in routine laboratory practice. Clin Lab Haem 2004; 26: 101–106.

[19] MATSUO T, KURIYAMA K, MIYAZAKI Y, YOSHIDA S, TOMONAGA M et al. Japan Adult Leukemia Study Group. The percentage of myeloperoxidase-positive blast cells is a strong independent prognostic factor in acute myeloid leukemia, even in the patients with normal karyotype. Leukemia 2003; 17: 1538–1543.

[20] MHAWECH P, BUFFONE GJ, KHAN SP, GRESIK MV. Cytochemical staining and flow cytometry methods applied to the diagnosis of acute leukemia in the pediatric population: an assessment of relative usefulness. J Pediatr Hematol Oncol 2001; 23: 89–92.

[21] NØRGAARD JM, OLESEN LH, OLESEN G, MEYER K, KRISTENSEN JS et al. FAB M4 and high CD14 surface expression is associated with high cellular resistance to Ara-C and daunorubicin: implications for clinical outcome in acute myeloid leukaemia. Eur J Haematol 2001; 67: 221–229.

[22] PAIETTA E. Expression of cell-surface antigens in acute promyelocytic leukemia. Best Pract Res Clin Haematol 2003; 16: 369–385.

[23] PEFFAULT DELATOUR R, LEGRAND O. MOREAU D, PERROT JY, BLANC CM et al. Comparison of flow cytometry and enzyme cytochemistry for the detection of myeloperoxydase in acute myeloid leukaemia: interest of a new positivity threshold. Br J Haematol 2003; 122: 211–216.

[24] STASI R, AMADORI S. AML-M0: A review of laboratory features and proposal of new diagnostic criteria. Blood Cells, Molecules, and Diseases 1999; 25: 120–129.

[25] SZCZEPANSKI T, VAN DER VELDEN VHJ, VAN DONGEN JJM. Classification systems for acute and chronic leukemias. Best Practice & Research Haematology 2003; 16: 561–582.

[26] TODISCO E, SUZUKI T, SRIVANNABOON K, COUSTAN-SMITH E, RAIMONDI SC et al. CD38 ligation inhibits normal and leukemic myelopoiesis. Blood 2000; 95: 535–542.

[27] TURGEON ML. Chemicals methods of cellular identification. Principles of special cytochemical stains. In: Salter MA, editor. Clinical Hematology. Theory and Procedures. 2nd edition. Little, Brown and Company, Boston Toronto, London 1993: 201–204.

[28] VENDITTI A, DEL POETA G, BUCCISANO F, TAMBURINI A, COX AC et al. Minimally differentiated acute myeloid leukemia (AML-M0): Comparison of 25 cases with other French-American-British subtypes. Blood 1997; 89: 621–629.

[29] WETZLER M, MCELWAIN BK, STEWART CC, BLUMENSON L, MORTAZAVI A et al. HLA-DR antigen-negative acute myeloid leukemia. Leukemia 2003; 17: 707–715.

[30] ZIEGLER-HEITBROCK HWL, ULEVITCH RJ. CD14: cell surface receptor and differentiation marker. Immunol Today 1993; 14: 121–125.