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Adult Acute Biphenotypic Leukemias: Polish Single Centre Experience

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Abstract

Biphenotypic AL (BAL) is characterised by presence of blasts which coexpress myeloid and T or B lineage antigens. In this study we describe the biological, clinical characteristic and outcome of 24 adult BAL patients treated in our center. We have analyzed a group of 480 patients with AL. To define BAL we used European Group for the Immunological Characterisation of Leukemias (EGIL) scoring system. Among whole group, 24 (5%) patients fulfilled the EGIL criteria of BAL. 22 patients were treated with conventional chemotherapy and in two cases allogeneic bone marrow transplantation from matched unrelated donors (MUD alloBMT) was performed. 50% of patients achieved complete remission. Ten patients died due to disease progression (no response after conventional therapy). In one case an extramedullary relapse after MUD alloBMT was a cause of death. Overall survival (OS) was 40%. Patients with BAL had poor outcome: induction of remission was difficult and overall survival was low.

Keywords: Biphenotypic leukemias; Diagnosis; Survival

Introduction

Acute leukemias (AL) are classified as myeloblastic or lymphoblastic, basing on blasts origin from appropriate cell line. In the French-American-British (FAB) classification combination of morphological and cytochemic staining of blast cells was sufficient to classify most of the acute leukemias. Advancement of knowledge and, above all, introduction of monoclonal antibodies in the widespread use of flow cytometry revealed that part of acute leukemias has the characteristics of both myeloid- and lymphoid- leukemia, and another part it is still difficult to define. This was reflected in the classification of the World Health Organization, which among this group of diseases distinguished undifferentiated leukemia, bilinear leukemia and biphenotypic leukemia [1]. Latest classification of tumors of hematopoietic and lymphoid tissue of the World Health Organization in 2008 introduced the term acute leukemia with mixed phenotype (mixed phenotype AL - MPAL) [2].

Acute biphenotypic leukemia (ABL) is characterized by the presence of blasts with the co-expression of antigens belonging to the myeloid and lymphoid lineage B and / or T cells. However, their mere presence is not enough to qualify a given leukemia as ABL. Originally there were distinguished subtypes called: acute lymphoblastic leukemia (ALL) with myeloid antigen and acute myeloid leukemia (AML) with positive lymphoid antigen, but it was important to distinguish "real" ABL from acute myeloid or lymphoid leukemia with one or two aberrant markers. To this end, in 1995 the European Group for the Immunological Characterization of Leukemias (EGIL) introduced a point system for defining BAL [3]. This system distinguished suitable markers belonging to the myeloid and lymphoid B and Tcells, while having the value on a scale of 0.5 to 2 points. According to this classification, ABL can be recognized when we reach a value of 2 points for the myeloid lineage, and 1 point for lymphoid lineage [3]. With these criteria estimated frequency of ABL, according to the literature, is about 5-10% [3-7]. It seems that the results achieved in the treatment of these patients are worse than in patients with well-defined AML or ALL.

The purpose of this study was immunophenotypig and clinical analysis of adult patients diagnosed with ABL and treated in the Department of Hematoloogy, Blood Neoplasms and Bone Marrow Transplantation of Wroclaw Medical University in Poland.

Material and Methods

Patients

The study group consisted of 480 patients diagnosed with acute leukemia and treated at the Department of Hematology, Blood Neoplasms and Bone Marrow Transplantation, Wroclaw Medical University. There were 272 men and 208 women. Median age of analyzed population was 56 years (range 18-84 years). All tests were performed at diagnosis, before starting of induction chemotherapy. We analyzed the results of bone marrow immunophenotyping performed at disease diagnosis.

Immunophenotyping and EGIL score

We analyzed the results of bone marrow immunophenotyping performed at disease diagnosis. For the selection of patients meeting the criteria for the ABL we used the EGIL score, which introduced a point system from 0.5 to 2 for the different antigens, depending on their linear specificity. Analysis was based on the general assumption that the marker is considered positive if more than 20% of the analyzed cells manifest its expression An exception was made for four high-specific antigens, ie.: cMPO, CD3, CD79 and TdT, for which the

limit is 10% positive cells. According to this classification, the ABL can be detected when the sum of the points for the myeloid lineage is more than 2, and for the lymphoid lineage 1, maintaining the specificity of the T- and B-line [3]. Details of the system EGIL point is shown in Table 1. Statistical analyses were performed using packet statistica 10.0.

Points	B-line	T-line	Myeloid line
2	CD79a	CD3	Anti-MPO
	cyt.IgM	Anti-TCRα/β	
	Cyt.CD22	Anti-TCRγ/δ	
1	CD19	CD2	CD13
	CD10	CD5	CD33
	CD20	CD8	CDw65
		CD10	
0.5	TdT	TdT	CD14
	CD24	CD7	CD15
		CD1a	CD64
			CD117

Table 1: EGIL (European Group for the Immunological Characterisation of Leukemia) scoring system for definition of adult acute biphenotypic leukemia (ABL).

Results

As a result of analysis we found the group of 24 patients who met the criteria for ABL (Table 2). In the group there were 14 women and 10 men, aged from 25 to 84 years (median 60 years). The majority of cases (23) were acute leukemia (AL) identified *de novo* and in one patient ABL was linked to previous myelodysplastic syndrome. We found coexistence of myeloid antigens and lymphoid T or B or, both T and B, respectively in 15, 4 and 5 cases. Before starting the treatment median white blood cells count (WBC)was 5.4 g/L (range 0.7 to 89.9); the average percentage of blasts in the bone marrow: was 57.17 ± 24.12 (range 23.5 to 98.0) with a median of 61.5%; the average percentage of blasts in peripheral blood: 35.65 ± 32.57 (range 0 to 93.0) with a median of 23.0%.

In 12 cases cytogenetic chromosome aberrations were detected, but there was no correlation between the presence of abnormal 3 patients had co-occurrence of a second cancer: lung, ovarian and colon cancer. 22 patients were treated according to the standards of the Polish Adult Leukemia Group (PALG) (protocols for ALL, AML and for AML over 60 years old, respectively in 5, 10 and 7 cases), 2 patients died before treatment. In 2 cases after conventional treatment allogeneic bone marrow from unrelated donor was performed. Example of patient's result is present on Figures 1a and 1b. 12 patients achieved complete remission (CR) of the disease, and 8 are still alive. 13 patients died due to lack of response to treatment or disease progression, and in one case, death occurred as a result of extramedullary relapse after allogeneic bone marrow transplantation from unrelated donor. Patients with ABL were characterized by statistically shorter survival than patients with acute myeloid leukemia and acute lymphoblastic leukemia (Figures 2 and 3).

	Patients	Sex	Age	EGIL Scores		
				B-Line	T-Line	Myeloid line
1	A.R.	M	42	6.0	2.5	2.0
2	B.Z.	F	84	0	4.0	3.5
3	B.J.	F	76	0	4.5	4.5
4	B.L.	F	48	0	2.0	3.0
5	C.E.	F	47	0	3.0	4.5
6	D.S.	M	80	0	2.0	4.5
7	F.K.	M	29	6.0	2.5	4.5
8	J.R.	M	64	3.0	0	4.0
9	K.A.	F	45	0	2.0	2.0
10	K.E.	F	51	3.0	0	3.5
11	K.J.	M	49	4.0	0	1.5
12	L.K.	F	42	4.0	3.5	2.0
13	O.A.	F	68	3.0	6.0	2.5
14	P.K.	F	67	4.0	0	2.0
15	P.T.	F	25	0	2.0	2.5
16	P.T.	M	76	0	3.5	2.0
17	P.J.	F	26	0	3.0	3.0
18	R.I.	F	67	0	6.5	3.0
19	S.E.	F	60	0	4.5	3.5
20	S.M.	F	57	0	4.0	5.0
21	S.R.	M	63	0	3.5	2.5
22	S.K.	M	39	2.0	2.0	4.0
23	W.B.	M	61	0	2.0	2.5
24	W.E.	F	49	0	2.0	2.0

Table 2: Patients characteristic according to EGIL scoring.

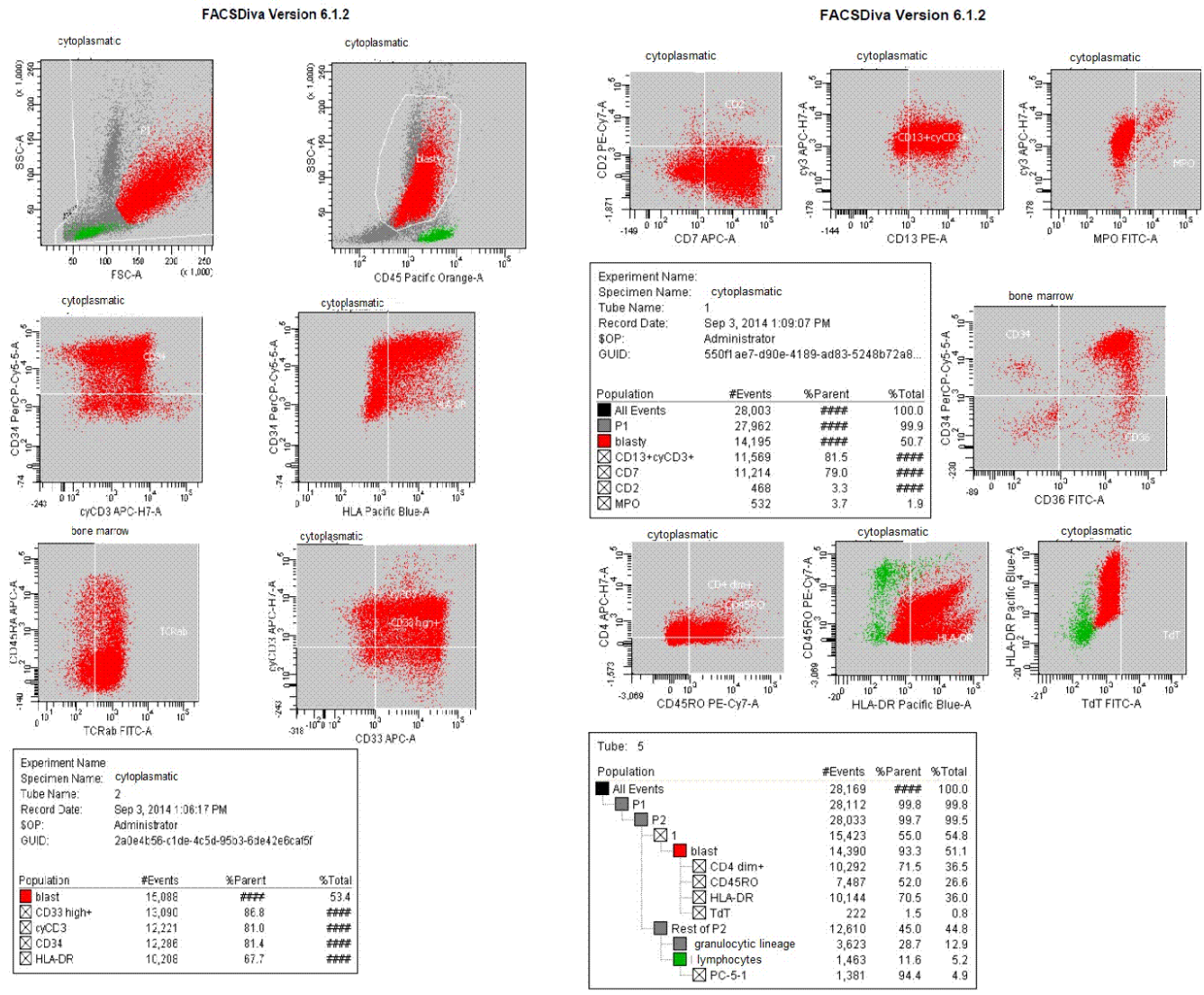

Figure 1: Example of immunophenotyping in patient with ABL.

Discussion

Approximately 5% of acute leukemias is difficult to classify because of coexpression of myeloid and lymphoid antigens. In order to clarify these problems so-called concept of “Unfaithful" and” mixed line "was discussed [4]. ABL can be diagnosed in adults as well as in children children under 2 years of age [5]. Most often it develops *de novo*, less frequently in the course of relapse (recurrence) of AML or ALL. ABL been described in literature as mixed or hybrid line leukemia: acute lymphoblastic leukemia antigen-positive myeloid (My + ALL) and acute myeloblastic leukemia lymphoid positive (Ly + AML). More frequent coexpression of myeloid and B-lymphoid than T-lymphoid is present Three-line coexpression is rare. This group includes biphenotypic leukemias and other leukemias with myeloblastic atypical expression of the individual markers of the line. Matutes et al. [6] proposed a scoring system that would allow ABL to be distinguish from those of antigen expression of another line:

1. For the line B the presence of CD79 antigen is required, detected as a transmembrane protein linked to immunoglobulins, which form part of a receptor for an antigen recognized on B cells and CD22 antigen.

2. For line T - the presence of CD3.

3. For the myeloid lineage - myeloperoxidase (MPO) detected with typical cytochemic or with use of monoclonal anti-MPO antibody.

There are no typical for ABL chromosomal alterations, although in 1/3 of the cases, the presence of chromosome Philadelphia (Ph+) and rearrangements in chromosome 11q23. There is need to create other markers with a high degree of specificity for the lines, for example: recognize chains of α/β and γ/δ receptor on T for T cell lines. ABL is

often similar to leukemia from old FAB classification: M1, M2, M4 and M5 less, but do not correspond to the type of leukemias M3, M6 and M7 [1,4].

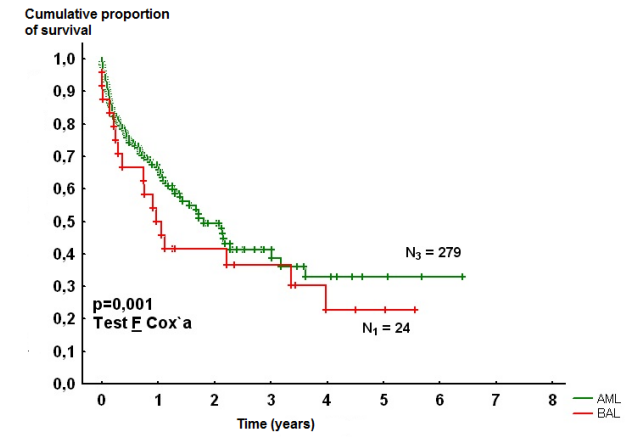

Figure 2: Survival of acute myeloid leukamia (AML) and acute biphenotypic leukemia (BAL) patients.

Hanson et al. [7] reported 52 (7%) cases of ABL from all 746 cases of acute leukemia in the period 1985-1990. The classification of these leukemias was based on the morphological evaluation, cytochemical staining and the use of flow cytometry. In these studies ABL was diagnosed on the basis of the antigens CD2, CD19, or CD20 on AML and CD11b-, CD13, CD14 and/or CD33 in ALL. Cytogenetic studies were also performed, in which the most common changes were confirmed: the presence of Ph + chromosome and 11q23 translocation, those two factor were found to be an independent prognostic factors. In addition, also other abbereations were found: t (8; 21), t (15; 17), inv (16) and trisomy 8. Carbonell et al. assessed 26 of 907 cases of ABL, which were divided into four groups depending on the immunophenotyping evaluation [8]. These groups were as follows:

1. Co-expression of myeloid antigens and line B
2. Co-expression of myeloid antigens and T lines
3. The three-line coexpression.
4. Co-expression of antigens line B and T.

Most rated leukemia showed the presence of Ph + chromosome and co-expression of myeloid antigens and line B. Ph+ has also been detected as isolated abnormality and other changes were found in chromosomes 1,6,9,10 and 19 [7].

Killick et al. conducted a retrospective study of 693 patients hospitalized between 1990 and 1997, where patients with ABL accounted for 3.6% of adults and children. In 15 patients there was coexpression of myeloid and B-lymphoid line markers, in 8 - myeloid and T-lymphoid line markers, in 1 - T and B and 1 with trilinear co-expression [9]. In 20 patients acute bilphenotypic leukemia was diagnosed *de novo*, and 5 ABL were diagnosed after initial treatment of AML or ALL. Authors used EGIL criteria. Induction therapy combined drugs as for AML protocol: cytarabine, etoposide, idarubicin for 5 days, and for ALL protocol: prednisone, vincristine, daunorubicin, and asparaginase. This treatment was ineffective and 25% of the patients died (early death). No deaths were observed early in the application of the induction for AML or ALL separately. Therefore, one type of therapy was recommended. Results of treatment of patients with secondary ABL were also bad. There is currently no consensus on the treatment of ABL. In the study of Zhang et al. [10] combined schedules of chemotherapy for myeloid and lymphoid leukemias were used. It has been shown that the use of combined patterns and for ALL was characterized by a higher number of CR than with AML protocols only (21, 11, and 6 cases, respectively). No statistically significant difference between the combination therapy and the scheme of AML was shown. As shown by our group, patients were treated using protocols for ALL, AML and for AML over 60 years of age (respectively: 5, 10 and 7 cases). No statistically significant differences in response to treatment depending on chemotherapy were observed. The study by Xu et al. demonstrated that in the group of 16 of 21 patients treated with combined chemotherapy or protocol for ALL 14 (88%) patients achieved CR [11]. But this group also received second induction cycle [11].

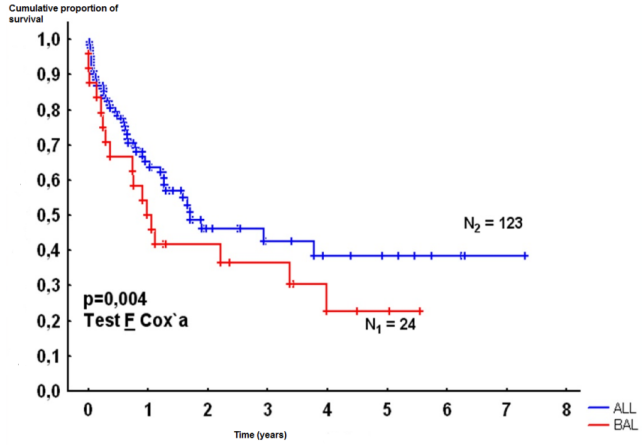

Figure 3: Survival of acute lymphoblastic leukamia (ALL) and acute biphenotypic leukemia (BAL) patients.

In our analysis, about 60% of blast cells expressed myeloid and B-lineage antigens and 30% had co-expression of myeloid and T-line antigens. In contrast, co-expression of markers of B, T, or three-line was rare. Most of the blasts had CD34 and HLA class II DR antigens expressed. Relapse of the disease occurred in 3 patients (21% of those who achieved remission), two of them were Ph +.

Important predictors for ABL are: age and the presence of Ph+ chromosome. Standard treatment is still to be established. Multicenter analysis could determine appropriate therapy. In the years 1986 - 1989 Sulak et al. evaluated the percentage of ABL, which was 6% [4]. On the leukemic cells there were present markers TdT, HLA-DR, CD19 and CD13, CD33, CD15. In the paper published by Zhang et al. [10] an analysis of 1693 patients with diagnosis *de novo* acute leukemia was carried out. In this group 51 (3%) of cases were ABL. These results were consistent with presented by Xu et al., where the reported incidence of ABL was of 1-8% [11]. Zhang et al. [10] confirmed the results of the predecessors [9] for the poor prognosis of patients with ABL, with two-year survival rate amounting to 39%, and four-year survival - to 8%.

In conclusion, acute biphenotypic leukemia in adults is a rare disease with poor prognosis. Few, so far published results [12] require

an expansion of research in a prospective multicenter analysis on a larger group of patients, to develop standards of diagnosis, treatment for prolong survival in patients with ABL.

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