Mixed-phenotype acute leukemia: suboptimal treatment when the 2008/2016 WHO classification is used

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Background
Different criteria have been used to diagnose mixed-phenotype acute leukemia (MPAL), which has impacted the number of individuals diagnosed with this pathology. Better outcomes have been reported when using acute lymphoblastic leukemia (ALL)-type chemotherapy in the treatment of MPAL.

Methods
We compared the outcome of 4 groups of patients with MPAL. Group 1 included patients diagnosed using the 2008/2016 World Health Organization (WHO) classification; group 2 included patients diagnosed using the European Group for the Immunological Characterization of Leukemias (EGIL) criteria; group 3 included patients diagnosed using either the EGIL or the 2008/2016 WHO criteria; and group 4 was comprised of patients diagnosed with MPAL using the EGIL classification only.

Results
We found a significantly worse disease-free survival (groups 1–4) and overall survival (OS) (groups 2 and 3) when comparing MPAL patients to other acute leukemia (AL) patients. A significantly better OS was obtained in patients (groups 2–4) treated with ALL-type chemotherapy compared to acute myeloid leukemia (AML)-type regimens.

Conclusion
In light of these results, and because a trend ($P=0.06$) was found with regard to a better OS in group 4 when compared to other AL patients, an argument can be made that the 2008/2016 WHO classification is underpowered to diagnose all MPAL cases, potentially resulting in the suboptimal treatment of some individuals with AL.

Key Words Mixed-phenotype acute leukemia, WHO classification, EGIL classification, Suboptimal treatment

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INTRODUCTION

The diagnosis and classification of acute leukemias (AL) rely on the implementation of immunophenotypic, immunohistochemical, morphological, cytogenetic, and molecular techniques [1, 2]. In the vast majority of cases, with the aid of these techniques, patients with AL can be classified and diagnosed with either acute lymphoblastic leukemia (ALL) or acute myeloid leukemia (AML) [3]. Nonetheless, in some instances, the blast population exhibits immunophenotypic and/or immunohistochemical features of more than one lineage (biphenotypic). The presence of two populations of blasts that belong to different lineages (bilineal) can also be observed [3]. Historically, these phenomena have been collectively defined as biphenotypic acute leukemias (BAL) [2].

In order to classify and diagnose BAL, in 1995, the European Group for the Immunological Characterization of Leukemias (EGIL) developed a scoring system, which was revised and modified in 1998 (Table 1) [2]. In 2001, the World Health Organization (WHO) adopted the classification proposed by the EGIL group, but due to certain caveats they proposed a new scoring system for this pathology

in 2008 (Table 2) [4, 5]. With this classification, leukemias formerly known as BAL were newly defined as mixed-phenotype acute leukemias (MPAL), and were included in the group of acute leukemias of ambiguous lineage [2, 4]. Additionally, the diagnosis of MPAL was simplified by including fewer but more specific markers [6]. In the new 2016 WHO classification of tumors of hematopoietic and lymphoid tissues, no major changes were introduced to the diagnosis of MPAL, except for cases in which it is possible to distinguish two distinct blast populations. In these patients, the diagnosis of MPAL is not based on the presence of specific markers, but only on the basis that each individual population would meet a definition for either B-cell, T-cell, or myeloid leukemia [7].

Based on the premise that aberrant markers are commonly expressed in certain leukemias, the 2008 WHO classification excluded certain cases even when the criteria for the diagnosis of MPAL were met. These cases include leukemias with recurrent genetic abnormalities, such as t(8;21), t(15;17), and inv(16), which should be categorized as AML with recurrent translocations [4]. This also applies to cases with fibroblast growth factor receptor 1 (FGFR1) mutations, chronic myeloid leukemia (CML) in blast crisis, AML with myelodysplasia-related changes, and therapy-related AML [8]. Furthermore, as worst outcomes have been reported with t(9;22)/*BCR-ABL1* and t(v;11q23)/*MLL* rearrangements in these patients [9, 10], the 2008 WHO criteria have identified these two entities as special categories in the classification of MPAL, with the remaining cases categorized as MPAL not otherwise specified (NOS) [2].

Similar outcomes have been reported among individuals diagnosed with MPAL according to the 2008 WHO criteria when compared to those diagnosed with BAL according to the EGIL classification. Nonetheless, fewer individuals are diagnosed with MPAL when the 2008 WHO criteria are used [6]. In terms of treatment regimens, different studies have demonstrated that these patients have a better outcome when ALL-type regimens are used [3]. Furthermore, due to the fact that fewer patients are diagnosed with MPAL when using the 2008 WHO classification, the implementation of this scheme has a foreseeable impact on therapeutic decision-making, and therefore potentially an impact on the outcome of some individuals [11].

The aim of this study is to compare the outcome of our patient population with MPAL/BAL when using both the EGIL and 2008/2016 WHO classifications. We also compare the outcome of these patients in light of different treatment regimens (ALL-type vs. AML-type regimens). Additionally, in an attempt to simplify the reading of this article, from now on, both BAL and MPAL cases will be referred to as MPAL.

MATERIALS AND METHODS

Patients

We retrospectively analyzed the data from 433 Mexican patients with AL, who were diagnosed and treated in the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ) in Mexico City, from January 2005 to June 2015. The patients were classified by using both the EGIL and 2008/2016 WHO criteria. Furthermore, based on the EGIL and 2008/2016 WHO classifications, we divided

Table 1. 1998 EGIL classification for biphenotypic acute leukemias (BAL).

Score	B-cell lineage	T-cell lineage	Myeloid lineage
2	cCD79a cIgM cCD22	cCD3/sCD3 anti-TCR α/β anti-TCR γ/δ	anti-MPO
1	CD19 CD10 CD20	CD2 CD5 CD8 CD10	CD13 CD33 CDw65 CD117
0.5	TdT CD24	TdT CD7 CD1a	CD14 CD15a CD64

To establish a BAL diagnosis, a score >2 must be established for at least two lineages.
Abbreviations: EGIL, European Group for the Immunological Characterization of Leukemias; TCR, T-cell receptor; MPO, myeloperoxidase; TdT, terminal deoxynucleotidyl transferase.

Table 2. 2008/2016 WHO criteria for the classification of mixed-phenotype acute leukemia (MPAL).

Lineage	Markers
Myeloid	Myeloperoxidase or Monocytic differentiation - at least two of the following markers: NSE, CD11c, CD14, CD64, lysozyme
T-cell	Cytoplasmic CD3 or Surface CD3
B-cell	Strong CD19 expression AND strong expression of at least one of the following markers: CD79a, cCD22, CD10 or Weak CD19 expression AND strong expression of at least two of the following markers: CD79a, cCD22, CD10

To establish an MPAL diagnosis, two or more lineages must be positive for the indicated antigens.
Abbreviations: WHO, World Health Organization; NSE, non−specific esterase.

the patients diagnosed with MPAL into 4 groups:

- Group 1: WHO (patients diagnosed using the 2008/2016 WHO classification).
- Group 2: EGIL (patients diagnosed using the EGIL criteria).
- Group 3: EGIL+WHO (patients diagnosed using either the EGIL or the 2008/2016 WHO criteria).
- Group 4: EGIL-WHO (patients included by the EGIL classification but excluded by the 2008/2016 WHO criteria).

Based on the new changes made by the 2016 WHO classification, we reassessed the potential diagnosis of new patients with MPAL. Patients who were diagnosed with MPAL using the 2008 WHO classification were also classified using the 2016 WHO criteria (group 1). The third group (EGIL+WHO) was added in order to assess the behavior of the patients diagnosed using either the EGIL or the 2008/2016 WHO criteria as a group. Additionally, the fourth group (EGIL-WHO) was added to evaluate if differences in the clinical endpoints exist between patients who were included by the EGIL classification but excluded by the 2008/2016 WHO classification, and patients diagnosed with other AL.

The following parameters were recorded: complete remission (CR), disease-free survival (DFS), and overall survival (OS). CR was assessed according to the criteria proposed by Cheson et al. [12]. DFS was defined as the interval between CR and relapse, while OS was defined as the length of time from the date of diagnosis to the last follow-up or date of death from any cause [13, 14].

Flow cytometry

In a 10-year period, erythrocyte-lysed blood, bone marrow, or both were processed and analyzed using flow cytometry with a mixed set of monoclonal antibodies. At first, a 5-color flow cytometry panel was used, followed by an 8-color panel, which included the following fluorochromes: phycoerythrin (PE), fluorescein isothiocyanate (FITC), peridinin chlorophyll (PerCP), allophycocyanin (APC), V450 and V500, PE and cyanine dye 7 (PE-Cy7) and PerCP-Cy5.5. The panel of antibodies included: CD2-FITC, cytoplasmic CD3 (cCD3-FITC), CD5-PE, CD7-APC, CD10-PE or CD10-APC, CD11b-Cy5, CD13-PE, CD14-PE, CD15-FITC, CD19-FICT, CD20-FITC or CD20-V450, CD22-PE or CD22-APC, CD33-PE or CD33-APC, CD34-PE, CD41-PE, CD56-FITC, CD61-FITC, CD64-PE or CD64-APC, cCD79a-PE, CD117-APC, CD235a-FITC, anti-MPO-FITC, HLA-DR-V450, IgM-PE or IgM-APC. The data were acquired and analyzed using a FACS Canto II flow cytometer (Becton Dickinson Immunocytometry Systems, San José, CA, USA) with the aid of FACSDIVA software (Becton Dickinson, San José, CA, USA). The samples were analyzed using a CD45 gating technique, which allowed analysis of only the blast population. Positive expression was defined when a marker was present in 10% or more of the blast population for cytoplasmic markers (cCD3, cCD79a, cIgM, and MPO) and CD34, 20% or more for myeloid markers and HLA-DR, and 30% or more for lymphoid markers [15, 16].

Cytogenetics

For all the individuals that participated in the study, conventional karyotype studies and fluorescence in situ hybridization (FISH) analysis for the *BCR-ABL* gene were carried out. Conventional karyotype analysis was performed on bone marrow (BM) blast cells after 24-48-hours of culture in tissue culture medium, according to standard techniques. A complex karyotype was defined by the presence of 3 or more chromosomal aberrations [10]. FISH analysis for the *BCR/ABL1* fusion gene was carried out using Vysis LS1 BCR-ABL dual-color dual-fusion probes (Vysis-Abbott, Maidenhead, UK), according to the instructions of the manufacturer and following standard techniques [17]. It is important to state that, due to economic constraints, polymerase-chain reaction (PCR) for the *BCR-ABL* translocation was not routinely performed.

Statistical analysis

A two-tailed Chi-square test and Fisher's exact test were used to compare categorical data between the groups. A Mann-Whitney test was used to compare non-normal data between the groups. A log-rank test and the Kaplan-Meier method were used in the analysis of survival parameters. A *P*-value ≤0.05 was considered statistically significant. Statistical analysis was performed using the SPSS software package, version 21.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Patient characteristics

Of the 433 patients, 8 (1.8%) were diagnosed with MPAL using the 2008/2016 WHO criteria (group 1), and 19 (4.4%) with MPAL using the EGIL classification (group 2). Moreover, 21 (4.9%) individuals were diagnosed with MPAL when using the EGIL+WHO criteria (group 3), and 13 (3.0%) with MPAL when using the EGIL-WHO classification (group 4).

Out of the 21 individuals in group 3, the cytogenetic analyses were available in 9 individuals (the blast cells arrested in metaphase of the cell cycle). Of these 9 patients, 5 had a normal karyotype, 1 had a complex karyotype, 1 presented with hyperdiploidy (48 chromosomes), and 1 had *BCR/ABL1* rearrangement. FISH analyses of the *BCR/ABL1* translocation were available in only 7 individuals, with only 1 patient presenting with this translocation (the same individual for whose cytogenetic analysis demonstrated the presence of the *BCR/ABL* rearrangement) (Table 3). The demographic and clinical characteristics of the patients with MPAL are shown in Tables 4 and 5.

Flow cytometric immunophenotyping

In patients diagnosed using the 2008/2016 WHO criteria (group 1), 87.5% (7/8) expressed B-lymphoid/myeloid antigens, while 12.5% (1/8) expressed T-lymphoid/myeloid antigens. In MPAL patients diagnosed using the EGIL criteria (group 2), 89.5% (17/19) expressed B-lymphoid/myeloid antigens, while 10.5% (2/19) expressed T-lymphoid/myeloid

Table 3. Clinical characteristics and endpoints of individual patients with MPAL.

Patient	Age (yr)	Gender	EGIL classification	2008/2016 WHO classification	Markers	Karyotype	FISH, t(9;22)	Treatment	HSCT	OS (mo)	DFS (mo)	CR
1	54	F	MPAL	B-cell ALL	B-L & M	NA	(-)	Hyper-CVAD	-	5.0	3.0	Yes
2	36	M	MPAL	MPAL	B-L & M	NA	NA	7+3	-	8.0	0.0	No
3	28	F	MPAL	MPAL	B-L & M	NA	(-)	PMHP	-	8.0	4.0	Yes
4	49	M	MPAL	MPAL	B-L & M	46,XY	(-)	Hyper-CVAD	-	14.0	8.0	Yes
5	33	M	T-cell ALL	MPAL	T-L & M	46,XY	(-)	Hyper-CVAD	-	12.0	1.0	Yes
6	79	F	MPAL	AML	B-L & M	NA	NA	Hyper-CVAD	-	8.0	6.0	Yes
7	25	M	MPAL	MPAL	B-L & M	48,XY,+10,+13	(-)	7+3+7	-	9.0	6.0	Yes
8	65	M	MPAL	T-cell ALL	T-L & M	NA	NA	7+3	-	1.0	0.0	No
9	39	M	MPAL	AML	B-L & M	NA	NA	7+3	+	6.0	2.0	Yes
10	69	F	MPAL	AML	B-L & M	NA	NA	Palliative care	-	0.1	0.0	No
11	42	F	MPAL	AML	B-L & M	46,XX,t(9;22)(q34;q11)	(+)	7+3	-	1.0	0.0	No
12	60	M	MPAL	AML	B-L & M	46,XY	NA	HOP 0195	-	16.0	9.0	Yes
13	33	M	MPAL	T-cell ALL	T-L & M	NA	NA	Hyper-CVAD	-	11.0	5.0	Yes
14	18	M	MPAL	B-cell ALL	B-L & M	NA	NA	Hyper-CVAD	-	5.0	3.0	Yes
15	61	M	B-cell ALL	MPAL	B-L & M	NA	NA	Palliative care	-	1.0	0.0	No
16	53	M	MPAL	B-cell ALL	B-L & M	NA	NA	7+3	-	0.2	0.0	No
17	21	M	MPAL	MPAL	B-L & M	46,XY	NA	Hyper-CVAD	-	11.0	9.0	Yes
18	74	M	MPAL	AML	B-L & M	46,XY	NA	Palliative care	-	5.0	0.0	No
19	18	M	MPAL	MPAL	B-L & M	Complex	(-)	Hyper-CVAD	-	4.0	0.0	No
20	46	M	MPAL	AML	B-L & M	46,XY	NA	Hyper-CVAD	-	1.0	0.0	No
21	51	M	MPAL	B-cell ALL	B-L & M	NA	NA	Hyper-CVAD	-	15.0	1.0	Yes

Abbreviations: EGIL, European Group for the Immunologic Characterization of Leukemias; WHO, World Health Organization; FISH, Fluorescence In Situ Hybridization; HSCT, allogenic hematopoietic stem cell transplantation; OS, overall survival; DFS, disease-free survival; CR, complete remission; MPAL, mixed-phenotype acute leukemia; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; B-L, B-lymphoid markers; T-L, T-lymphoid markers; M, myeloid markers; CVAD, Cyclophosphamide+Vincristine+Adriamycin+Dexamethasone; 7+3, Cytarabine+idarubicin; PMHP, Princess Margaret Hospital Protocol; NA, not available.

Table 4. Demographic and clinical characteristics of patients with MPAL (Group 1 and 2).

	WHO (Group 1)				EGIL (Group 2)			
	ALL (N=216)	AML (N=209)	MPAL (N=8)	P	ALL (N=213)	AML (N=201)	MPAL (N=19)	P
	Median (range)				Median (range)			
Age, years								
Median (range)	28.5 (16–85)	48 (15–88)	30.5 (18–61)	<0.001	28 (16–85)	48 (15–88)	46 (18–79)	<0.001
≥65, patients (%)	11 (5.1)	55 (26.3)	-		10 (4.7)	52 (25.9)	4 (21.1)	
Gender				0.137				0.137
Male, patients (%)	121 (56)	110 (52.6)	7 (87.5)		119 (55.9)	105 (52.2)	14 (73.7)	
Hemoglobin, g/dL	8.15 (5–15.4)	8.4 (3.4–14.8)	8 (6.9–14.3)	0.328	8.19 (5–15.4)	8.4 (3.4–14.8)	8 (5.3–12.7)	0.366
Leukocytes, ×10⁹/dL	6.75 (0.1–422.9)	7.6 (0.2–353)	4.7 (0.7–82.3)	0.513	6.8 (0.1–422.9)	8.1 (0.2–353)	2.86 (0.2–97.9)	0.183
Platelets, ×10⁹/dL	38.5 (4–380)	36 (0–1,123)	30 (15–253)	0.643	39 (5–380)	36 (0–1123)	22 (4–253)	0.031
Blasts in PB (%)	60 (1–96)	32 (1–98)	54 (13–62)	<0.001	58 (1–96)	32.5 (1–98)	19.5 (2–92)	0.001
Blasts in BM (%)	68.9 (7–100)	39 (2–97)	41.2 (28– 94)	<0.001	68 (7–100)	37 (2–97)	48 (19–94)	<0.001
CR, patients (%)	143 (66.2)	94 (45)	5 (62.5)	<0.001	140 (65.7)	91 (45.3)	11 (57.9)	<0.001
OS, months	12 (0–123)	9 (0–114)	8 (1–14)	0.285	12 (0–123)	9 (0–114)	8 (0.13–16)	0.036
DFS, months	9 (0–93)	18 (0–109)	6 (0–9)	<0.001	9 (0–93)	18 (0–109)	6 (0–9)	<0.001

Abbreviations: WHO, World Health Organization; EGIL, European Group for the Immunological characterization of Leukemias; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; MPAL, mixed-phenotype acute leukemia; PB, peripheral blood; BM, bone marrow; CR, complete remission; OS, overall survival; DFS, disease-free survival.

antigens. With the EGIL+WHO classification (group 3), 85.7% (18/21) and 14.3% (3/21) of these patients expressed B-lymphoid/myeloid and T-lymphoid/myeloid antigens, respectively. Finally, with the EGIL-WHO criteria (group 4), 84.6% (11/13) and 15.4% (2/13) of these individuals expressed B-lymphoid/myeloid and T-lymphoid/myeloid anti-

Table 5. Demographic and clinical characteristics of patients with MPAL (Group 3 and 4).

	EGIL+WHO (Group 3)				EGIL-WHO (Group 4)			
	ALL (N=210)	AML (N=202)	MPAL (N=21)	P	ALL (N=210)	AML (N=202)	MPAL (N=13)	P
	Median (range)				Median (range)			
Age, years								
Median (range)	28 (16–85)	48 (15–88)	46 (18–79)	<0.001	28 (16–85)	48 (15–88)	53 (18–79)	<0.001
≥65, patients (%)	10 (4.8)	52 (25.9)	4 (19)		10 (4.8)	52 (25.7)	4 (30.8)	
Gender								0.469
Male, patients (%)	116 (55.2)	106 (52.5)	16 (76.2)	0.115	116 (55.2)	106 (52.5)	9 (69.2)	
Hemoglobin, g/dL	8.14 (5–15.4)	8.4 (3.4–14.8)	8 (5.3–14.3)	0.319	8.14 (5–15.4)	8.4 (3.4–14.8)	8 (5.3–11.4)	0.344
Leukocytes, ×10^9/dL	6.8 (0.1–422.9)	8.25 (0.2–353)	2.86 (0.2–97.9)	0.188	6.8 (0.1–422.9)	8.25 (0.2–353)	2.86 (0.2–97.9)	0.229
Platelets, ×10^9/dL	39 (5–380)	36 (0–1,123)	23 (4–253)	0.039	39 (5–380)	36 (0–1123)	19 (4–11)	0.03
Blasts in PB (%)	58 (1–96)	33 (1–98)	23 (2–92)	0.001	58 (1–96)	33 (1–98)	19.5 (2–92)	0.001
Blasts in BM (%)	69.8 (7–100)	38 (2–97)	49.5 (19–94)	<0.001	69.8 (7–100)	38 (2–97)	51 (19–93)	<0.001
CR, patients (%)	139 (66.2)	91 (45)	12 (57.1)	<0.001	139 (66.2)	91 (45)	6 (36.2)	<0.001
OS, months	13 (0–123)	9 (0–114)	8 (0.13–16)	0.02	13 (0–123)	9 (0–114)	5 (0.13–16)	0.06
DFS, months	9 (0–93)	18 (0–109)	6 (0–9)	<0.001	9 (0–93)	18 (0–109)	5 (0–9)	0.001

Abbreviations: WHO, World Health Organization; EGIL, European Group for the Immunological characterization of Leukemias; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; MPAL, mixed-phenotype acute leukemia; PB, peripheral blood; BM, bone marrow; CR, complete remission; OS, overall survival; DFS, disease-free survival.
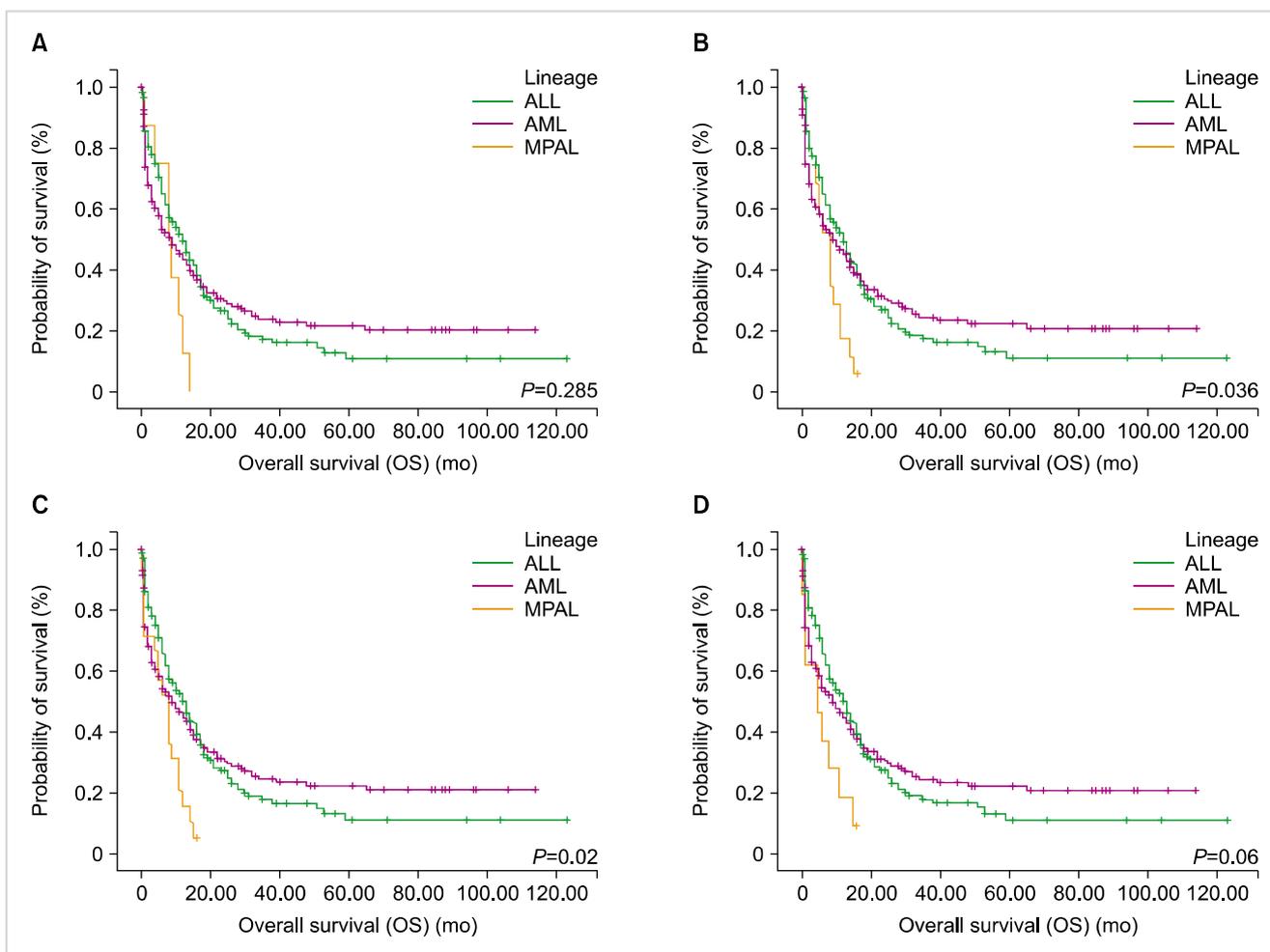

Fig. 1. Overall survival of mixed-phenotype acute leukemia patients versus individuals with other acute leukemias in group 1 (**A**), group 2 (**B**), group 3 (**C**) and group 4 (**D**).
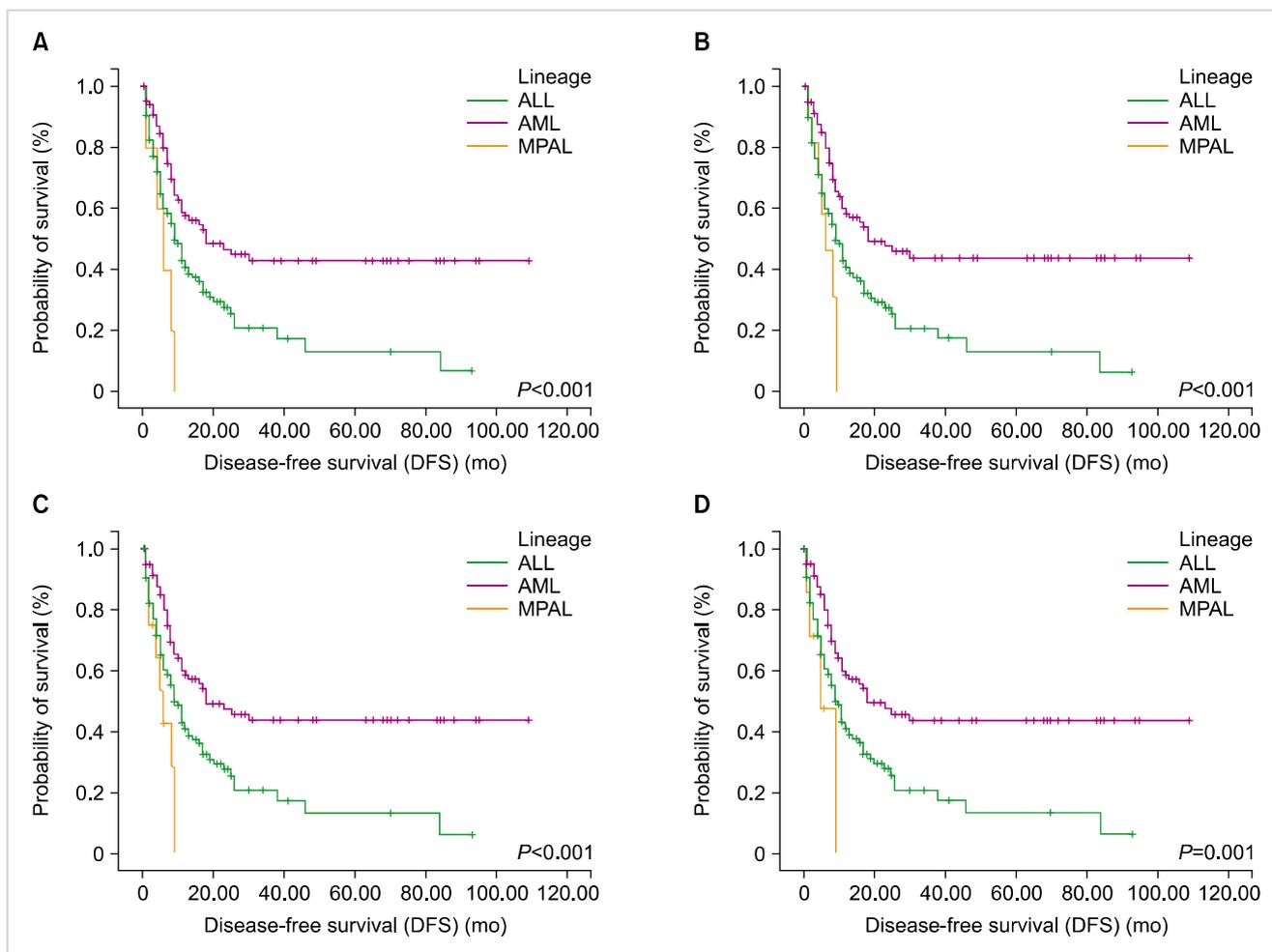
Fig. 2. Disease-free survival of mixed-phenotype acute leukemia patients versus individuals with other acute leukemias in group 1 (**A**), group 2 (**B**), group 3 (**C**) and group 4 (**D**).

gens, respectively.

Treatment of ALL and AML patients

Based on the WHO classification, the administered treatment was as follows: of the 216 ALL patients, 196 (90.7%) received induction therapy, while 20 (9.3%) received palliative care, primarily due to their low functional status (ECOG; Eastern Cooperative Oncology Group) at diagnosis. Moreover, of the 209 AML patients, 159 (76%) received induction therapy, while 50 (24%) received palliative care for the same reasons as ALL patients.

CR rate

Of the individuals diagnosed according to the 2008/2016 WHO and the EGIL classifications (groups 1 and 2), 62.5% (5/8) and 57.9% (11/19) achieved CR, respectively. Additionally, with the EGIL+WHO and EGIL-WHO criteria (groups 3, 4), 57.1% (12/21) and 46.2% (6/13) of the patients achieved CR, respectively. As seen in tables 4 and 5, in all 4 groups we found a significantly higher CR rate in the patients diagnosed with MPAL than in those diagnosed with AML. However, the rate was lower than that of ALL individuals.

OS and DFS

The patients diagnosed with MPAL according to the 2008/2016 WHO criteria (group 1) achieved median OS of 8 months, which was not significantly different from that of individuals diagnosed with ALL (12 mo) or AML (9 mo) (P=0.285). On the other hand, with this classification, the median DFS for MPAL (6 mo) was significantly lower than that of ALL (9 mo) and AML (18 mo) ($P<0.001$). With the EGIL classification (group 2), the median OS and DFS (8 mo and 6 mo, respectively) of MPAL patients were significantly lower than those of patients with ALL (12 mo and 9 mo, respectively) and AML (9 mo and 18 mo, respectively) (P=0.036 for OS, $P<0.001$ for DFS). For the EGIL+WHO criteria (group 3), the median OS and DFS (8 mo and 6 mo, respectively) were significantly lower for MPAL than those for ALL (13 mo and 9 mo, respectively) and AML (9 mo and 18 mo, respectively) (P=0.02 for OS, $P<0.001$ for DFS). For the EGIL-WHO classification (group 4), the median DFS (5 mo) was significantly lower for MPAL than those for ALL (9 mo) and AML (18 mo), while difference of median OS showed no statistical significance (P=0.06 for OS, P=0.001 for DFS) (Fig. 1, 2) (Table 4, 5).

Table 6. Clinical endpoints of MPAL patients according to different treatment regimens.

	WHO (Group 1)			EGIL (Group 2)			EGIL+WHO (Group 3)			EGIL-WHO (Group 4)		
	ALL-type N=5	AML-type N=2	P	ALL-type N=11	AML-type N=6	P	ALL-type N=12	AML-type N=6	P	ALL-type N=8	AM-type N=4	P
CR, patients (%)			1			0.109			0.107			0.067
Yes	4 (80)	1 (50)		9 (81.8)	2 (33.3)		10 (83.3)	2 (33.3)		7 (87)	1 (25)	
No	1 (20)	1 (50)		2 (18.2)	4 (66.7)		2 (16.7)	4 (66.7)		1 (13)	3 (75)	
OS, months												
Median (range)	11 (4–14)	8 (8–9)	0.356	11 (1–16)	1 (0.23–9)	0.024	11 (1–16)	1 (0.23–9)	0.013	11 (1–16)	1 (0.23–6)	0.009
DFS, months												
Median (range)	4 (0–9)	6 (0–6)	0.774	8 (0–9)	2 (0–6)	0.194	8 (0–9)	2 (0–6)	0.295	5 (0–9)	2 (0–2)	0.307

Abbreviations: EGIL, European Group for the Immunologic Characterization of Leukemias; WHO, World Health Organization; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CR, complete remission; OS, overall survival; DFS, disease-free survival.

Treatment impact on CR, OS, and DFS in MPAL

Out of the 21 individuals in group 3, 18 received a curative chemotherapies (due to poor performance status, palliative care was applied to the other 3 patients). Of these 18 patients, 12 received ALL-type regimen and 6 AML-type regimen. Finally, only 1 patient underwent an allogeneic hematopoietic stem cell transplant (HSCT) because of costs or lack of donors (Table 3).

In groups 1–4, no statistically significant correlation was found in the CR and DFS between ALL-type regimen group and AML-type regimen group. However, in the EGIL (P=0.024), EGIL+WHO (P=0.013), and EGIL-WHO (P=0.017) groups (groups 2–4), ALL-type regimen group had a higher median OS compared to AML-type regimen group. On the other hand, patients classified using the WHO criteria (group 1) had no statistically significant differences for median OS between ALL-type regimen group and AML-type regimen group (Table 6).

DISCUSSION

In 26 patients with EGIL-defined MPAL, Heesch *et al.* [18] found an overexpression of stem cell-associated genes, and these genes linked to leukemogenesis (i.e. *BAALC, ERG,* and *MN1*). Thus, it was hypothesized that MPAL may originate from stem/progenitor cells harboring both myeloid and lymphoid markers [18]. Furthermore, like other studies, the majority of our MPAL patients were men and expressed a B-lymphoid/myeloid immunophenotype [9, 10, 18-20].

As stated above, the 2008 WHO criteria are more stringent and exclusive than the EGIL classification, and thus, more patients are diagnosed with the latter. In 452 adult Chinese patients with AL, Xu *et al.* [19] determined that 4.6% had MPAL according to the EGIL classification, whereas in another retrospective Chinese study of 4,780 patients (aged 14–81 yr) with AL, it was determined that 2.4% of individuals had MPAL according to the 2008 WHO criteria [20]. Additionally, a Dutch study of 518 adult and pediatric patients with AL found that 5.8% of patients were diagnosed with MPAL using the EGIL criteria, while only 1.5% of

patients were diagnosed with MPAL using the 2008 WHO classification [11]. In our study, these differences are well established; 4.4% of individuals were diagnosed with MPAL according to the EGIL classification, while 1.8% were diagnosed with MPAL using the 2008/2016 WHO criteria.

In terms of clinical endpoints, worse outcomes have been reported in adult patients diagnosed with MPAL using either the EGIL or the 2008 WHO criteria, compared to individuals diagnosed with AML or ALL [9, 19, 21]. In all 4 groups in our study, the DFS was significantly shorter in MPAL patients than those of AML or ALL patients. When the OS was compared between MPAL patients and AML or ALL patients, the MPAL patients had a worse OS in all 4 groups. Nonetheless, only in the EGIL and EGIL+WHO categories (group 2, 3), the differences were statistically significant.

When comparing therapeutic outcomes between ALL-type and AML-type regimens in MPAL individuals diagnosed using either the EGIL or the 2008 WHO criteria, better outcomes have been reported in patients treated with ALL-type regimen [10, 18, 22, 23]. Furthermore, MPAL patients treated with combined AML+ALL-type regimen showed similar remission rates despite the possibility of greater toxicity, compared to MPAL patients treated with only ALL-type regimen [9, 20, 22, 24]. Thus, it is recommended that these patients should be treated with ALL-type regimen with the addition of tyrosine kinase inhibitor in the case of t(9;22)-positive MPAL [3, 25]. Moreover, if possible, an allogenic HSCT should be performed in adult patients with this leukemia because of better outcomes reported in several studies [23, 26].

As shown in table 6, MPAL patients treated with ALL-type regimen had better OS in comparison to those treated with AML-type regimen. Statistically significant differences were found in the EGIL, the EGIL+WHO, and the EGIL-WHO classifications (groups 2–4); whereas in the 2008/2016 WHO classification (group 1), no statistically significant differences were found in OS between ALL-type regimen and AML-type regimen.

Group 4 (EGIL-WHO) patients showed shorter DFS (P=0.001) and trend towards worse OS (P=0.06), compared to ALL or AML patients. In addition, ALL-type regimens

led to significantly better outcome in these patients. Therefore, it can be assumed that patients in the EGIL-WHO category (group 4) have similar behavior to MPAL diagnosed with other criteria (group 1–3), rather than AML or ALL patients. Likewise, other studies have revealed poor survival parameters and distinct clinical behavior in this group, compared to other AL patients. For example, Al-Seraihy et al. [27] identified poor outcome in pediatric patients who were initially diagnosed as MPAL with the EGIL criteria but subsequently reclassified as ALL with 2008 WHO criteria. Furthermore, OS of some t(8;21) patients diagnosed as MPAL with EGIL criteria was found to be shorter than that of AML patients with the same translocation [19, 22, 28].

In an attempt to differentiate MPAL from other ALs at molecular level, several studies have assessed the gene expression of the leukemic blasts in patients with this entity. Different gene expression patterns have been found between MPAL and other ALs when using the EGIL or the 2008 WHO criteria [18]. However, contrary to previous studies, de Leeuw et al. [29] found that microRNA expression profiles categorized AL of ambiguous lineage as either AML or ALL, rather than distinct group.

Due to the rarity of this type of AL, no prospective clinical trials were reported. Therefore, it is required to conduct prospective multicenter clinical trials in order to evaluate clinical behavior and preferable chemotherapeutic regimen in patients diagnosed with MPAL according to the EGIL and the 2008/2016 WHO classifications. Ideally, in these trials, two arms should be present; one that evaluates ALL-type regimens in patients in group 4 [EGIL-WHO], and another that evaluates the same patients in light of the chemotherapeutic regimen that should be administered according to the assignation of each individual using the 2008/2016 WHO classification. Moreover, if possible, comprehensive molecular genetic analysis should be performed at the time of diagnosis in order to clarify whether MPAL cases can be differentiated from other AL using certain molecular markers, and stratify the outcome of patients with MPAL based on the presence of certain molecular abnormalities [30].

Due to the retrospective nature and the small number of patients with MPAL of this study, these results have to be interpreted with caution. Nonetheless, because group 4 (EGIL-WHO) patients showed clinical behavior distinct from that of AML or ALL patients but similar to other MPAL patients, an argument can be made that the 2008/2016 WHO MPAL classification is underpowered to diagnose all MPAL cases, potentially resulting in the suboptimal treatment of some individuals with AL. Therefore, we argue that the EGIL classification is still a useful scheme that should be considered when diagnosing patients with MPAL.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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