Mixed-phenotype acute leukemia: state-of-the-art of the diagnosis, classification and treatment

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Mixed-phenotype acute leukemia (MPAL) is a heterogeneous group of hematopoietic malignancies in which blasts show markers of multiple developmental lineages and cannot be clearly classified as acute myeloid or lymphoblastic leukemias. Historically, various names and classifications were used for this rare entity accounting for 2-5% of all acute leukemias depending on the diagnostic criterias used. The currently valid classification of myeloid neoplasms and acute leukemia published by the World Health Organization (WHO) in 2016 refers to this group of diseases as MPAL. Because adverse cytogenetic abnormalities are frequently present, MPAL is generally considered a disease with a poor prognosis. Knowledge of its treatment is limited to retrospective analyses of small patient cohorts. So far, no treatment recommendations verified by prospective studies have been published. The reported data suggest that induction therapy for acute lymphoblastic leukemia followed by allogeneic hematopoietic cell transplantation is more effective than induction therapy for acute myeloid leukemia or consolidation chemotherapy. The establishment of cooperative groups and international registries based on the recent WHO criterias are required to ensure further progress in understanding and treatment of MPAL. This review summarizes current knowledge on the diagnosis, classification, prognosis and treatment of MPAL patients.

Key words: mixed-phenotype acute leukemia, MPAL, diagnosis, classification, prognosis, treatment

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INTRODUCTION

Acute leukemia (AL) is a heterogeneous group of neoplastic hematopoietic diseases caused by malignant transformation of hematopoietic stem cells. Differentiation of malignant transformed cells ceases at the level of leukemic blasts and their subsequent uncontrolled proliferation and accumulation in organs, in particular bone marrow, leading to clinical manifestations of the disease[1]. The diagnosis and classification of AL require a multidisciplinary approach integrating morphology, immunophenotyping, cytogenetics and molecular biology in the presence of ≥ 20% of blasts in bone marrow aspirates[2,3]. Based on the above mentioned examinations, most AL cases may be classified as either acute myeloid leukemia (AML) or acute lymphoblastic leukemia (ALL). A small heterogeneous group of ALs with blasts showing markers of multiple developmental lineages is referred to as mixed-phenotype acute leukemia (MPAL) (ref.[4,5]). Depending on the diagnostic criteria used and patient's age distribution, MPAL accounts for 2-5% of all AL cases[2,6]. The name and diagnostic criteria of this rare disease group have undergone several changes over a period of time. Despite the advances in examination methods and updated criteria, the diagnosis and classification of MPAL continue to be challenging.

DIAGNOSTIC CRITERIA AND CLASSIFICATION OF MPAL

Mixed-phenotype leukemia was first mentioned in the 1980s when monoclonal antibodies started to be used in the classification of leukemic cells[7]. Routine application of monoclonal antibodies showed that a small proportion of AML and ALL express aberrant immunophenotypic markers. It was necessary to develop diagnostic criteria for this AL subgroup, referred to as biphenotypic. The first diagnostic criteria for the so-called biphenotypic acute leukemia (BAL) were laid down in 1991. BAL were distinguishable from other ALs not only by their biological nature but also prognosis. The cytochemical and immunophenotypic markers characteristic for individual developmental lineages were assigned 0.5 to 2 points according to their specificity. The most specific markers with the highest points were cytoplasmic CD3, CD22 and myeloperoxidase (MPO) for the T-lymphoid, B-lymphoid and myeloid lineages, respectively. Acute leukemia with a total of 2 or more points in different developmental lineages were classified as biphenotypic[8].

In 1995, the diagnostic criteria for BAL, as previously set by Catovsky et al. 4 years ago, became the basis for recommendations for immunological classification of AL proposed by the European Group for the Immunological Characterization of Leukemias (EGIL). According to the EGIL criteria, BAL was defined as achieving a score of

more than 2 for the myeloid and/or lymphoid lineages. These criteria included novel, highly specific markers such as CD79a. The threshold for marker positivity when a monoclonal antibody is used was set at 20% of positive blasts; the exceptions were highly specific markers such as MPO, CD79a, CD3 and terminal deoxynucleotidyl transferase, with only 10% positivity being sufficient[9]. In 1998, the EGIL criteria were revised again and CD117 was added as a highly specific marker for the myeloid lineage[10]. The 1998 EGIL criteria for BAL are clearly summarized in Table 1.

The 2008 WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues, as well as an older 2001 WHO classification, replaced the EGIL criteria, using a simpler diagnostic algorithm derived from fewer specific markers[4]. Moreover, the new classification also changed the name of the entity from BAL to MPAL. The currently widely used 2008 WHO diagnostic criteria (Table 2), also included in a 2016 revision, are based on the expression of surface/cytoplasmic CD3 specific for the T-lymphoid lineage and expression of MPO and/or monocytic differentiation specific for the myeloid lineage. For MPO positivity, no minimum level of expression has been set. Cytochemical studies of MPO require positivity in more than 3% of blasts[11]. Cases of otherwise typical B-ALL with homogeneous expression of lymphocyte markers and only isolated weak MPO positivity, as determined by immunophenotyping methods, should not be classified as B/myeloid MPAL (ref.[5]). Monocytic differentiation is defined by the presence of at least two of the following markers: nonspecific esterase, CD11c, CD14, CD64 or lysozyme. In the case of the B-lymphocytic lineage, no characteristic antigen is defined; therefore, strongly expressed CD19 and the presence of at least one of the other B markers (CD10/CD22/CD79a) are required. With weak CD19 expression, the presence of at least two other B markers is necessary[4]. Based on co-expression of markers from different developmental lineages, MPAL is divided into B/myeloid, T/myeloid and rare B/T lymphoblastic or trilineage subgroups. To make a diagnosis of MPAL according to the 2008 WHO criteria, a recommended minimum range of the following monoclonal antibodies should be used: anti-CD3, anti-CD19 and 3 other B-specific markers (CD22, CD79a, CD10), anti-MPO (cytochemical and with flow cytometry) and 2-3 markers associated with the monocytic lineage (CD14, CD11c, CD64, CD36 or anti-lysozyme) (ref.[6]). The presence of an aberrant nonspecific marker from another developmental lineage is not sufficient to meet the diagnostic criteria for MPAL. Given their high prevalence, both the 2008 WHO classification and the 2016 revision defined individual subgroups of MPAL with the presence of the Ph1 chromosome or *BCR/ABL1* rearrangement and MPAL with t(v;11q23) and rearrangement of the mixed-lineage leukemia (*MLL*) gene[4,5]. Acute promyelocytic leukemia, AML with recurrent cytogenetic abnormalities, blast-phase chronic myeloid leukemia or Ph1+ ALL are not considered as MPAL even if they meet the immunophenotypic diagnostic criteria. Compared to immunophenotyping, cytogenetic findings are regarded as

Table 1. Diagnostic criteria for biphenotypic acute leukemia published by the EGIL in 1998 (ref.[10]).

Number of points	B-lymphoid lineage	T-lymphoid lineage	Myeloid lineage
2	CD79a	CD3 (m/cyt)	anti-MPO
	cyt IgM	anti-TCR α/β	(anti-lysozyme)
	cyt CD22	anti-TCR γ/δ	
1	CD19	CD2	CD13
	CD10	CD5	CD33
	CD20	CD8	CDw65
		CD10	CD117
0.5	TdT	TdT	CD14
	CD 24	CD1a	CD15
		CD7	CD64

cyt - cytoplasmic, m - membrane, MPO - myeloperoxidase, TCR - T-cell receptor, TdT - terminal deoxynucleotidyl transferase

Table 2. Diagnostic criteria for mixed-phenotype acute leukemia according to the WHO revision of classification 2016 (ref.[5]).

Lineage	Markers
Myeloid	myeloperoxidase or monocytic differentiation (at least two of the following markers: NSE, CD11c, CD14, CD64 or lysozyme)
T-lymphoid	cytoplasmic or surface CD3
B-lymphoid	strong expression of CD19 and at least one of the following markers: CD79a, cytoplasmic CD22, CD10; or weak expression of CD19 and at least two of the following markers: CD79a, cytoplasmic CD22, CD10

NSE - nonspecific esterase

Table 3. Categories of MPAL according to the 2008 WHO classification[4].

Category	Definition
MPAL with t(9;22) (q34;q11.2); *BCR-ABL1*	acute leukemia meeting the criteria for MPAL, with blasts carrying translocation (9;22) or *BCR-ABL1* rearrangement
MPAL with t(v;11q23); *MLL* rearrangement	acute leukemia meeting the criteria for MPAL, with blasts carrying translocation involving the *MLL* gene
MPAL, B/myeloid, NOS	acute leukemia meeting the criteria for B-lymphoid and myeloid lineages of MPAL, with blasts not carrying genetic abnormalities involving *BCR-ABL1* or *MLL*
MPAL, T/myeloid, NOS	acute leukemia meeting the criteria for T-lymphoid and myeloid lineages of MPAL, with blasts not carrying genetic abnormalities involving *BCR-ABL1* or *MLL*
MPAL, NOS	acute leukemia meeting the criteria for B- and T-lymphoid lineages or trilineage MPAL
Other entities classified as MPAL	NK-cell lymphoblastic leukemia/lymphoma acute bilineal leukemia

NOS - not otherwise specified

more important for the diagnosis of individual nosological entities[2]. Similarly, secondary AML with myelodysplastic features and therapy-related AML are excluded from the MPAL group. In the valid 2016 revision of the WHO classification, bilineal leukemia defined by the presence of different blast populations are added to mixed-phenotype leukemia. If 2 or more blast populations are present, individual populations must meet the diagnostic criteria for B-, T-lymphoblastic or myeloid leukemia[5]. The relationship between bilineal and biphenotypic leukemia is unclear; two populations of leukemic cells may only represent subclones of a single pluripotent leukemic cell. However, this fact generally does not influence the diagnostic or therapeutic approach to the disease[4,12]. Rare is the so-called acute undifferentiated leukemia (AUL), a group of diseases with blast cells expressing no specific marker allowing classification into the myeloid or lymphoid lineages. In AUL, no more than one marker of the developmental lineage is usually expressed; however, the cytoplasmic markers CD3, CD22, CD79a and MPO are always negative[13]. A special subgroup of MPAL comprises rare entities such as NK-cell lymphoblastic leukemia/lymphoma[4]. The MPAL categories as defined by the 2008 WHO criteria are listed in Table 3.

CHARACTERISTICS OF MIXED-PHENOTYPE LEUKEMIA

Incidence

Mixed-phenotype acute leukemia is a rather rare disease. The prevalence of MPAL among AL patients depends on the diagnostic criterias used. The retrospective meta-analysis of 7627 cases of AL in both pediatric and adult populations reported 213 (2.8%) and 119 (1.6%) cases of MPAL according to the EGIL and 2008 WHO criteria, respectively[2]. Among 4780 patients aged 14 or more presenting with *de novo* AL, Chinese authors noted 117 (2.4%) cases of MPAL as defined by the 2008 WHO criteria[14]. In an analysis of 633 children younger than 14 years, 24 (3.8%) and 11 (1.7%) cases of MPAL meeting the EGIL and 2008 WHO criteria were found[15]. Another investigation of 1855 AL cases in children, reported 35 (1.9%) and 19 (1.0%) MPAL cases, respectively[16]. A British study of 100 patients with MPAL suggested that the incidence was higher in adults than in children[6]. Finally, MPAL was found to be more prevalent in males[3,6,14]. Selected characteristics of samples of patients with MPAL meeting the 2008 WHO criteria as reported in the literature are summarized in Table 4.

Clinical manifestations

The signs of MPAL patients are similar to those seen in patients with other types of AL, including anemic syndrome, hemorrhage and infection[2]. Studies have shown that MPAL is more frequently associated with CNS involvement as compared with AML and ALL (ref.[17,18]). The involvement of the CNS at presentation was noted in 5 (18.5%) cases among 27 children with MPAL diagnosed according to the EGIL criteria and in 2 (18.2%) out of 11 children meeting the 2008 WHO criteria[15]. More frequent extramedullary (in particular CNS) involvement was also reported in 8 (38.1%) out of 21patients with MPAL as defined by the EGIL in another study[19]. A hepatosplenomegaly and lymphadenomegaly were more common in MPAL than in AML (ref.[20]).

Morphology

Based on morphological assessment of blasts, most MPAL patients may be classified into the AML or ALL groups. The peripheral blood and bone marrow smears in 90 patients diagnosed with MPAL according to the WHO criteria in the British study revealed 39 (43%) cases of AML meeting the French-American-British criteria and

Table 4. Overview of selected samples of patients with MPAL (according to the 2008 WHO criteria).

Patient sample		Matutes et al. (ref.[6])	Munker et al. (ref.[27])	Yan et al. (ref.[14])	Weinberg et al. (ref.[21])
Epidemiology	Cases of AL	n.s.	n.s.	4780	n.s.
	Cases of MPAL	100	95	117 (2,4%)	45
	Age (median)	n.s.	1-68 (20)	14-81 (35)	1-78 (28)
	Males (%) / females	62 (62%) / 38	49 (52%) / 46	60 (51%) / 57	25 (56%) / 20
Immunophenotype	B/Myeloid	59 / 100 (59%)	55 (58%)	64 / 117 (55%)	30 / 45 (67%)
	T/Myeloid	35 / 100 (35%)	31 (33%)	38 / 117 (32%)	14 / 45 (31%)
	Other	6 / 100 (6%)	9 (9%)	15 / 117 (13%)	1 / 45 (2%)
Cytogenetics	NK	10 / 76 (13%)	32 (34%)	33 / 92 (36%)	14 / 32 (44%)
	CK	24 / 76 (31%)	n.s.	22 / 92 (24%)	n.s.
	Ph1+	15 / 76 (20%)	10 (11%)	14 / 92 (15%)	5 / 32 (16%)
	MLL	6 / 76 (8%)	10 (11%)	4 / 92 (4%)	2 / 32 (6%)
	Other	21 / 76 (28%)	n.s.	19 / 92 (21%)	11 / 32 (34%)
Morphology	AML	38 / 90 (42%)	n.s.	40 / 117 (34%)	n.s.
	ALL	39 / 90 (43%)	n.s.	51 / 117 (44%)	n.s.
	AUL	13 / 90 (15%)	n.s.	26 / 117 (22%)	n.s.

NK - normal karyotype, CK - complex karyotype, AUL - acute undifferentiated leukemia, n.s. - not stated, AL - acute leukemia, AML - acute myeloid leukemia, ALL - acute lymphoblastic leukemia

38 (42%) cases of ALL. The remaining 13 (14%) cases had different population of blast cells and were diagnosed as having AUL (ref.[6]). Among another group of 117 patients, morphology results suggested ALL in 51 (44%) cases, AML in 40 (34%) and AUL in 26 (22%) (ref.[14]). Heterogeneous morphological findings were also reported in a group of 35 pediatric patients with MPAL meeting the EGIL criteria. The morphology of ALL, AML and AUL was observed in 12 (34%), 7 (21%) and 14 (41%) patients. In two cases, two blast populations were noted, suggesting the diagnosis of bilineal leukemia[16].

Immunophenotypic findings

Among MPAL patients, B/myeloid is the most common immunophenotype[6,14,21,22]. Out of 100 patients included in the British study, B/myeloid, T/myeloid and B/T lymphoblastic immunophenotypes were identified in 59 (59%), 35 (35%) and 4 (4%) individuals, respectively. Two cases (2%) were found to have trilineage coexpression (B/T/myeloid). There were no statistically significant differences in the presence of B/myeloid, T/myeloid and B/T lymphoblastic immunophenotypes with respect to age, gender or morphology[6]. Other study of 117 patients reported 64 (55%) cases of B/myeloid, 38 (33%) of T/myeloid and 14 (12%) of B/T lymphoblastic immunophenotypes. A trilineage immunophenotype was observed in only one patient. Strongly positive expression of CD34, typical for hematopoietic stem cells, found in 84% of MPAL patients confirms the theory that mixed-phenotype leukemia develops from a pluripotent stem cell able to differentiate into both myeloid and lymphocyte progenitors during the development of acute leukemia[14]. In another group of 45 patients with MPAL meeting the 2008 WHO criteria, CD34 positivity was found in 31 (69%) cases[21]. In a study of 24 children with MPAL diagnosed according to the EGIL criteria, the distribution of immunophenotypes was as follows: B/myeloid in 14 (58%), T/myeloid in 7

(29%), B/T lymphoblastic in 2 children and trilineage in 1 patient. In a subgroup of 11 patients meeting the 2008 WHO criteria, positive CD34 expression was observed in 10 (91%) children. Thirteen patients meeting only the EGIL criteria were classified as having ALL according to the 2008 WHO criteria[15].

Cytogenetics

Cytogenetic abnormalities may be seen in 59-91% of MPAL patients[23]. For MPAL, no pathognomonic cytogenetic change has been identified[2]. Frequent cytogenetic findings include the Ph1 chromosome and *MLL* rearrangement, with their presence being a prerequisite for classification into individual MPAL subgroups[4,5]. In 76 patients with available cytogenetic results, British investigators found the presence of the Ph1 chromosome and *MLL* rearrangement in 15 (20%) and 6 (8%) MPAL cases, respectively[6]. The Ph1 chromosome was reported in 14 (15%) and *MLL* rearrangement in 4 (4%) out of 92 patients with available cytogenetic findings in another MPAL study[14]. The two above mentioned patient groups met the 2008 WHO diagnostic criteria. Among 24 pediatric patients with MPAL diagnosed in accordance with the EGIL criteria, only one case of Ph1 positivity (Ph+) was noted and changes in the *MLL* gene were found in three individuals[15]. Another study reported chromosomal abnormalities in 29 out of 33 children with MPAL diagnosed according to the EGIL criteria, with 4 (12.1%) patients having rearrangement of the *MLL* gene[16]. While MPAL with Ph1 chromosome positivity is more frequently seen in the elderly, *MLL* rearrangement is more common in pediatric patients[6,15,16]. A complex karyotype (CK), considered an adverse prognostic factor, was reported in 32% and 24% of patients in two biggest studies[6,14]. The presence of a CK, associated with myelodysplastic syndrome (MDS), was suggested to be an exclusion criterion in study by Weinberg et al.; according to the 2008 WHO

criteria, the patients should be classified as having AML with myelodysplastic changes[4,21].

Molecular biology

Molecular genetic changes in MPAL have been studied in only very small numbers of patients. Their association with prognosis and treatment still remain unclear. Mutations of genes typical for leukemic cells were described in 12/31 (39%) patients with MPAL in Chinese study[14]. *FLT3* mutation was reported in 7/15 (45%) patients with T/myeloid MPAL, with internal tandem duplication being the mechanism in 6/7 (86%) cases[24].

Prognostic factors

Mixed-phenotype acute leukemia is considered a condition with adverse prognosis[6]. Given its low incidence, only limited information on treatment outcomes is available in the literature. The published data suggest poor treatment outcomes, in terms of both the probability of achieving complete remission and overall survival (OS), as compared with AML or ALL (ref.[19,25]). A study of treatment in 35 children with MPAL meeting the EGIL criteria showed results comparable to those in AML but worse overall survival than in pediatric ALL (ref.[16]). Differences in treatment outcomes between pediatric and adult patients with MPAL were investigated in the British study, stating that the median overall survival was significantly longer in children than in adults (139 vs. 11 months) (ref.[6]). Similar results were noted in another study reporting longer overall survival in patients younger than 21 years of age[21]. No significant differences in overall survival were observed between patients with B/myeloid and T/myeloid MPAL (ref.[16,21]). The median survival was significantly different with respect to cytogenetic findings: 8 months for Ph1+ MPAL, 139 for a normal karyotype (NK) patients and 28 months for patients with other cytogenetic abnormalities (except for a CK and *MLL* rearrangement) (ref.[6]). Poor prognosis of patients with Ph1+ MPAL was also confirmed by other studies[19,26]. The prognosis of patients is also affected by the selection of induction therapy, with induction therapy for ALL being more beneficial than that for AML, as well as of consolidation therapy, with better outcomes seen in patients undergoing allogeneic hematopoietic cell transplantation (HCT) (ref.[6,14,15,22]).

TREATMENT OF MPAL

Given the low incidence, little has been published on the treatment of MPAL. As of now, both guidelines and prospective studies concerning treatment of MPAL patients are missing. The published data suggest better treatment outcomes resulting from induction therapy for ALL and combined AML/ALL-like protocols followed by allogeneic HCT (ref.[6,14-16,22]). In a study of 35 children with MPAL meeting the EGIL criteria, remission was achieved in 12/23 (52%) patients receiving AML induction therapy and 10/12 (82%) children treated according to an ALL protocol. With reinduction therapy according to the ALL protocol, remission was achieved in 8/10 (80%) patients who initially failed to respond to AML therapy[16]. Another study reported the achievement of complete remission in all 11 children with MPAL diagnosed according to the 2008 WHO criteria using an ALL-like induction therapy[15]. Complete remission achieved by 23/27 (85%) patients after chemotherapy for ALL and by 14/34 (41%) individuals after induction therapy for AML was noted in the British study[6]. In a study of 66 subjects with MPAL according to 2008 WHO criteria, complete remission was observed in 29/41 (71%) patients receiving combined AML/ALL induction therapy, 10/16 (63%) patients receiving ALL therapy and 4/9 (44%) individuals receiving AML-induction therapy. The 3-year overall survival differed significantly between the HCT and chemotherapy-only treated subgroups (77% vs. 16%) (ref.[22]). The overall survival of patients undergoing allogeneic HCT was reported to be significantly longer than in case of those receiving consolidation chemotherapy (22 vs. 9 months) (ref.[14]). In another cohort of 95 allogeneic HCT patients with MPAL, the three-year OS of 69% and relapse incidence of 29% were reported. No difference in survival was observed between patients who underwent HCT in first or second CR (ref.[27]). When selecting adequate therapy, all available information should be included such as patientage, comorbidities, clinical condition and their primary disease (morphology, cytogenetics, immunophenotyping). Cytogenetic tests may reveal Ph1+ patients with translocation (9;22) – the Philadelphia chromosome. Identification of these patients is important for selecting therapy different from that for Ph1-negative patients. Treatment of Ph1+ MPAL is derived from recommendations for treating Ph1+ ALL which, until the era of tyrosine kinase inhibitors (TKIs) was thought to have rather adverse prognosis[25,28]. One retrospective analysis compared treatment outcomes of 13 patients with Ph1+ MPAL and 27 patients with Ph1+ ALL. The results showed comparable probability of achieving complete remission (100% vs. 85%) as well as the overall 5-year survival (55% vs. 53%) in both groups[29]. Therapy for Ph1+ MPAL is based on an ALL protocol adjusted to the patient's age, combined with TKI and followed by allogeneic HCT (ref.[25]). In patients with Ph1+ ALL, excellent short-term outcomes were achieved using dasatinib combined with corticosteroids and intrathecal chemotherapy[30]. It is assumed that MPAL arising from a hematopoietic stem cell (with strong CD34+ expression and a potential to differentiate into various lineages) will not be curable by chemotherapy alone[19,20,25]. As in Ph1+ ALL, AML arising from MDS or with adverse cytogenetics, incurable without allogeneic HCT, the role of transplantation in the first complete remission is similar in mixed-phenotype leukemia[25-28]. The use of a protocol for ALL with a broader spectrum of cytostatic drugs in the first cycle of chemotherapy seems to be more reasonable than of protocols for AML therapy[15,25]. Patients who fail to achieve remission after AML induction therapy should receive reinduction therapy according to an ALL protocol[16]. In samples of MPAL patients reported in the literature, cases of CNS involvement are relatively fre-
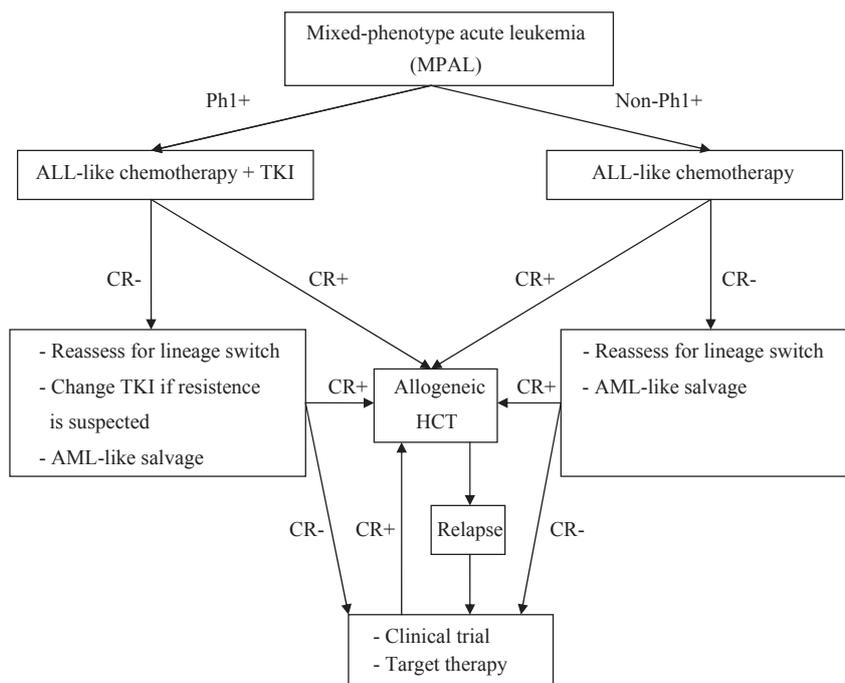
Fig. 1. Algorithm for treating patients with MPAL.

quent; these should be treated according to an adequate ALL protocol[17,18,25]. It is recommend that in patients with a white blood cell count of more than 100×10^9/L or those with the Ph1 chromosome, intrathecal chemotherapy should be supplemented by prophylactic irradiation of the CNS (ref.[15]). The algorithm for treating MPAL patients is shown in Fig. 1.

DISCUSSION

Acute leukemias represent a diverse group of malignant blood disorders with various laboratory and clinical presentation. The majority of AL could be classified according to the antigenic profile of the blasts as AML or ALL. However, some cases of AL were found to express both lymphoid and myeloid lineage-specific antigens. Historically, various terms and classifications have been used to describe this rare entity. The most recent WHO classification from 2016 terms the disease as MPAL and categorizes it together with AUL to entity called AL with ambiguous lineage[4,5]. Information about MPAL is very limited because of the rarity and various diagnostic criteria used historically. MPAL seems to be more frequent when EGIL criteria are used, as they evaluate higher number of less-specific antigens[5,10]. Some cases of MPAL that fulfil EGIL criteria do not meet the recent WHO ones[15,21]. In the large population-based study MPAL make up only 0.6 % of all AL in comparison to previously reported 2-5% (ref.[31]). Median age at diagnosis varies in series between 20 and 35 years with bimodal age distribution[14,21,27]. The reason is probably due to biology and differentiation potential into mixed-lineage leukemic cells in leukemogenesis of childhood ALL or AML more common in the elderly population. In most MPAL case

series male patients predominated similarly to AML or ALL in large population-based studies[3,6,14,21,27,32]. Future research of genetic and hormonal characteristics, environment exposures are likely to provide insight into the observed sex differences.

The diagnosis of MPAL always relies on immunophenotyping followed by cytogetics which allows us to exclude AML with defined recurrent cytogenetic abnormalities[4,5]. It is recommended to incorporate lineage specific antigens advised by WHO into routine examinations not to underdiagnose MPAL (ref.[5,6,13]). The morphology can reveal the dysplastic background of AL with suspicion of AML with myelodysplasia-related changes, but cannot differentiate MPAL from AML or ALL. In the case of bilineal leukemia 2 distinct blast populations can be recognized by morphology.

Clinical features of MPAL are similar to other AL as the biology of the disease, infiltration of bone marrow with suppression of hematopoiesis, are the same[1]. Extramedullary involvement (particularly CNS) is reported to be more frequent in comparison to ALL or AML. This difference can be partly explained by a relatively higher occurrence of hyperleukocytosis at the diagnosis[27].

The molecular biology background and its prognostic importance in MPAL is not well known, but may contribute to risk-stratification and treatment management. When a diagnosis of MPAL is established, search for Ph1 chromosome or *BCR/ABL1* translocation is indicated as these indicate the the treatment strategy - addition of TKI to treatment regiments. Similarly to Ph1+ ALL complete molecular remissions can be achieved even in elderly MPAL patients with only a combination of prednisolone and TKI (ref.[28-30,33]). The presence of *FLT3/ITD* in MPAL can be a potential therapy target for inhibitors similarly to AML (ref.[34]). ALL-like or AML/ALL combined

treatment regiments appear to be more effective than AML-like treatments consisting of a limited number of cytostatics. When drugs such as cytarabin, etoposide and antracyclines with activity against AML and vincristine, antimetabolites and corticosteroids with anti-ALL activity are used in rotation, higher CR rates are achieved[6,15,16]. CNS prophylaxis/treatment should be a mandatory part of treatment strategy[15,18]. Reported results from case series indicate that allogeneic HCT could be a prospective treatment option for patients achieving CR compared with chemotherapy-only regiments[14,22,27]. Younger age is generally considered to be a favorable prognostic factor as it is associated with fewer comorbidities allowing use of an intensive treatment approach consisting of high dose chemotherapy followed by allogeneic HCT. Moreover, cytogenetic abnormalities considered to be unfavorable are also less frequent in younger age[6,15,16]. Patients not responding to the first induction chemotherapy course should be offered re-induction containing cytostatics with different lineage activity as reported [15,16]. The role of allogeneic HCT is similar to other AL with non-favorable prognosis, when MPAL patients undergoing HCT have similar results to AML or ALL (ref.[27]). A more critical approach to HCT is in childhood MPAL, as reported long-term results are similar in transplanted and chemotherapy-only groups, suggesting indication of allogeneic HCT only in patients failing to achieve molecular or immunologic remission (< 0.01% blast cells) after induction therapy[15,16]. Taken together, treatment results have improved in the last decades as allogeneic HCT and TKI have became part of the treatment . However, the treatment and prognosis of MPAL remain unsatisfactory with need forimprovement.

CONCLUSION

Mixed-phenotype acute leukemia represents a rare group of hematopoietic malignancies in which blast cells show markers of multiple developmental lineages at the same time. The low prevalence of this disease will require the establishment of international registries and cooperative groups to ensure further progress in the diagnosis, classification and treatment of MPAL patients. The results of prospective studies are expected to contribute to the development of guidelines and improvement in the generally poor prognosis of MPAL.

Search strategy and selection criteria
Our research strategy was aimed at evaluating papers reporting on the diagnosis, classification, prognosis and treatment strategies of MPAL. Scientific articles from 1991 to 2016 were searched using the PubMed database. All searches were up to date as of November 2016. The search terms used included "mixed phenotype acute leukemia", "acute leukemia of ambiguous origin", " acute biphenotypic leukemia" and "acute bilineal leukemia". Only English and Czech language peer-reviewed papers were investigated.

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