RESEARCH ARTICLE

Editorial Process: Submission:11/08/2022 Acceptance:04/24/2023

Clinical and Molecular Characteristics of Patients with Mixed Phenotype Acute Leukemia

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Abstract

Introduction: Mixed phenotype acute leukemia (MPAL) is a rare heterogeneous disease with a poor prognosis. This study analyzed the clinical, immunophenotypic, molecular, and cytogenetic characteristics of a group of patients with MPAL. **Methods:** This prospective study included 75 patients diagnosed with MPAL according to the World Health Organization (WHO)-2016 diagnostic criteria, using cytochemistry, conventional cytogenetics, and molecular studies. Screening of BCR::ABL1 fusion gene was performed by Fluorescent in-situ hybridization (FISH) and polymerase chain reaction (PCR). **Results:** Children represented 49.3% of MPAL patients. The main phenotype was B-lymphoid/myeloid (80%). Molecular alterations were detected in 17 patients (22.7%). The BCR::ABL1 fusion gene was detected in 10 patients (13.3%).. Myeloid protocols were used to treat 58 patients (77.3%), and lymphatic protocols in 17. By the end of the follow-up, 57 patients (76%) achieved complete remission (CR). There was no association between BCR::ABL1 and response to treatment. The cumulative overall survival (OS) at 12 months was 47.8%. The bone marrow transplantation (BMT) was associated with better OS ($p = 0.027$). The disease-free survival (DFS) was not affected by all tested prognostic factors. **Conclusion:** MPAL is a complex entity with heterogeneous features. BCR::ABL1 is a common abnormality. BMT is associated with better OS.

Keywords: Mixed Phenotype Acute Leukemia- MPAL- Cytogenetic Analysis- BCR::ABL1- KMT2A

Asian Pac J Cancer Prev, **24 (4)**, 1217-1223

Introduction

Mixed phenotype acute leukemia (MPAL) is acute leukemia of ambiguous lineage (ALAL) that does not fit within lineage-specific leukemia categories (Gerr et al., 2010). It is a rare disease that comprises 2% to 5% of all acute leukemias (Weinberg et al., 2014). MPAL is a high-risk class with a poor prognosis. Survival proportions of 15-35% were reported before the transplant era (Shi and Munker, 2015).

In a large study multicenter study including 519 adult patients diagnosed with de novo MPAL who underwent allo-SCT and were reported to the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation (EBMT), the overall survival was 56.3% at three years (Munker et al., 2017).

MPAL can be biphenotypic presenting cross-lineage myeloid, B-lymphoid, T-lymphoid antigen expression, or bilineal with a distinct single-lineage blast population (Weinberg and Arber, 2010). The term MPAL was introduced in the World Health Organization (WHO) classification in 2008. This classification was updated in 2016 by adding subcategories, i.e., MPAL with t(9;22)(q34;q11.2) BCR::ABL1 fusion gene and MPAL with t(v;11q23.3) KMT2A-rearranged (KMT2A-r) (Swerdlow et al., 2017).

BCR::ABL1 fusion gene (Philadelphia chromosome) resulting from the t(9;22)(q34;q11.2) translocation can be detected in chronic myeloid leukemia (CML), acute lymphoblastic leukemia (ALL), or acute myeloid leukemia (AML) (Soverini et al., 2019). It encodes a tyrosine kinase, p190, p210, or p230, promoting cell proliferation and suppressing apoptosis (Ayatollahi et al., 2018).

Given that there is no consensus on a therapeutic strategy to assign patients with MPAL to lymphoid- or myeloid-directed treatment, a better understanding of the characteristics of MPAL is crucially needed. This study aimed to analyze the clinical, immunophenotypic, molecular, and cytogenetic characteristics of a group of MPAL patients as defined according to WHO-2016 criteria.

Materials and Methods

This prospective study included newly diagnosed patients with MPAL from January 2016 to December 2018. All patients were recruited from the outpatient clinics of the pediatric and medical oncology departments of the National Cancer Institute (NCI), Cairo University, Egypt. Patients with incomplete data were excluded

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from the analysis. All patients or patients´ guardians had provided informed consent to share in scientific research on admission. The study was performed following the 2013 declaration of Helsinki and was approved by the institutional review board of the NCI.

The diagnosis of MPAL was based on WHO diagnostic criteria (Béné and Porwit, 2012), morphological examination of peripheral blood (PB) and bone marrow (BM) smears, cytochemistry, conventional cytogenetics, and molecular studies. BCR::ABL1 screening performed by FISH and PCR. Eighty patients were diagnosed as MPAL based on immunophenotyping, but five having blasts with a mixed phenotype and t(8;21) were excluded later because they would be reclassified as having AML, according to 2016 WHO classification (Swerdlow et al., 2017).

Analytical Methods

Flow Cytometry

Peripheral blood and BM samples were tested for immunophenotyping. A complete panel of monoclonal antibodies was done, including myeloid markers MPO, CD13, CD33, CD117, CD64, CD15, CDIIB, CD36, CD14; B-cell markers CD19, CD10, CD22, CD20, cytoplasmic m, cytoplasmic CD79a, kappa, lambda; T-cell markers cytoplasmic CD3, CCD7, CD1, CD2, CD4, CD8, CD56; other markers CD45, CD11C, CD34, NG2, MHC CLASS II and CD135. Analysis was done on six colors Navios cytometer (Beckman Coulter Diagnostics, USA). Minimal residual disease (MRD) was assessed on days 14, 28, and 42 post-induction.

Cytogenetic analysis

Following the standard techniques, pre-treatment diagnostic BM samples were subjected to conventional karyotyping using G-banded metaphase cells from unstimulated 24-hour cultures. In most cases, at least 20 metaphases were analyzed using an IKAROS imaging system (Metasystems, Altlußheim, Germany). The karyotypes were interpreted using the International System for Human Cytogenetic Nomenclature (ISCN 2016). Fluorescence in situ hybridization (FISH) was performed following the manufacturer's instructions.

For detecting BCR::ABL1 fusion gene, LSI BCR-ABL1 dual color dual fusion probe (Vysis, Abbott, Maidenhead, UK) was used. A minimum of 10 metaphases and 200 interphase nuclei were analyzed using a fluorescence microscope (AxioImager.Z1 mot, Carl Zeiss Ltd., Hertfordshire, UK) equipped with appropriate filter sets. Image capture and processing were performed using an ISIS imaging system (Metasystems, Altlußheim, Germany).

Molecular detection of fusion gene transcripts

According to the manufacturer's instructions, total RNA was extracted from bone marrow or peripheral blood samples using Qiagen RNA Blood Mini Kit (cat#5723, Germany). RNA was reverse transcribed using a high-capacity complementary DNA reverse transcription kit (cat#4368814, USA) Applied biosystems. Reverse transcription-polymerase chain reaction (RT-PCR) was performed for all samples for the detection of fusion transcripts t(9;22)(q34;q11) p190 and p210, t(4;11) (q21;q23), t(12;21)(p13;q22), t(1;19) (q23;p13) and t(8;21)(q22;q22), according to the BIOMED-1 guidelines.

Treatment

Lymphatic protocol

All pediatric patients received total XV protocol (modified from St. Jude total XV protocol), while adults received German Multicenter Study Group for Adult Acute Lymphoblastic Leukemia (GMALL) protocol. Risk classification to low, standard, and high risk was based on initial presenting features. Response to treatment was based on the minimal residual disease (MRD) at specific time points (day 15 and day 42 from the start of induction chemotherapy). The treatment protocol consists of three phases, induction of remission, consolidation, and maintenance. The induction phase (42 days) involved four-drug regimens (prednisone, vincristine, doxorubicin, and L-asparaginase), the consolidation therapy (8 weeks) consisted of 4 cycles of high-dose methotrexate, and the maintenance phase (120 weeks for females and 146 weeks for males).

Patients with t(9;22) BCR-ABL1 started tyrosine kinase inhibitor (imatinib) at a dose of 260 mg/m^2 per day once molecular results were available and continued till the end of treatment. If the patient had an MRD < 0.1% by flow cytometry at the end of induction and more than 3 log reduction (major molecular response), or MRD PCR at week 7, the patient was not considered eligible for hematopoietic stem cell transplantation. Allogeneic hematopoietic stem cell transplantation is indicated for patients with high-risk leukemia (poor response to induction treatment; MRD > 1%).

Myeloid Protocol

All patients received standard induction chemotherapy with 3+7 protocol (idarubicin as a short infusion for three days with cytarabine 100 mg/m2 continuous infusion for seven days). Patients, who achieved CR, according to their risk stratification, were offered consolidation with high dose cytarabine and HLA matching followed by allogeneic bone marrow transplantation. Refractory cases received re-induction with a high-dose cytarabine-based regimen. Response to treatment was based on MRD at specific time points (day 14 and day 28 from induction chemotherapy). The outcome of the induction treatment was assessed at day 28, where patients were categorized into complete remission (CR) or refractory group.

Statistical analysis

Statistical analysis was done using IBM© SPSS© Statistics version 25 (IBM© Corp., Armonk, NY, USA). Numerical data were expressed as mean and standard deviation or median and range as appropriate. Qualitative data were expressed as frequency and percentage. Chi-square test (Fisher's exact test) was used to examine the relation between qualitative variables. For quantitative data, comparison between two groups was made using independent sample t-test or Mann-Whitney test. Survival analysis was done using the Kaplan-Meier method, and

comparison between two survival curves was made using the log-rank test. A p-value < 0.05 was considered significant.

Results

Out of 75 patients, 37 (49.3%) were 18 years old or younger, i.e., children. Manifestations of BM failure were noticed in 36 patients (48%). Fifty-six patients (74.7%) had hypercellular BM at presentation. Sixty patients (80%) had B-lymphoid/myeloid type. Baseline demographic, clinical, and laboratory characteristics are shown in Table 1.

Table 1. Baseline Demographic, Clinical, and Laboratory Characteristics of the Studied Group

Parameter		Value
Age (years)		19 (1-63)
	Children	37 (49.3%)
	Adults	38 (50.7%)
Sex	Male	56 (74.7%)
	Female	19 (25.3%)
Clinical Manifestations	Hepatomegaly	31 (41.3%)
	Splenomegaly	30 (40.0%)
	Lymphadenopathy	33 (44.0%)
	Central nervous system infiltration	9 (12.0%)
	Fever	39 (52.0%)
Laboratory Characteristics	Total leukocytic count ($x10^3$/mL)	28.0 (1.0-883.2)
	Hemoglobin concentration (gm/dL)	8.0±2.4
	Platelet count ($x10^3$/mL)	48.0 (5.0-393.0)
	Peripheral blood blast cells (%)	25.0 (0.0-98.0)
	Bone marrow blast cells (%)	80.0 (22.0-99.0)
Bone marrow cellularity	Normocellular	19 (25.3%)
	Hypercellular	56 (74.7%)
Immunophenotyping	B-lymphoid/myeloid	60 (80.0%)
	T-lymphoid/myeloid	15 (20.0%)
Myeloid Markers	MPO	74 (98.7%)
	CD33	70 (93.3%)
	CD13	69 (92.0%)
B-cell Markers	CD19	57 (76.0%)
	CD10	58 (77.3%)
	CD22	49 (65.3%)
T-cell Markers	CD7	21 (28.0%)
	CD2	16 (21.3%)
	CD3	15 (20.0%)
Molecular alterations	BCR::ABL1 fusion	10 (13.3%)
	KMT2A rearrangements	3 (4.0%)
	t (12;21)	2 (2.7%)
	JAK2 V617F mutation	1 (1.3%)
	FLT3-ITD mutation	1 (1.3%)
Modal chromosome number	Diploid	22/37 (59.5%)
	Hypodiploid	3 /37 (8.1%)
	Hyperdiploid	12/37 (32.4%)
Structural Chromosomal abnormalities	Yes	22/37 (59.5%)
	No	15/37 (40.5%)
Chromosome 21 abnormalities	Yes	15/37 (40.5%)
	No	22/37 (59.5%)

Data are expressed as median (range), mean±SD, or number (%)

All patients with T/myeloid phenotype were positive for cytoplasmic CD3. Molecular alterations were detected in 17 patients (22.67%). Using the FISH technique, BCR::ABL1 was seen in 10 patients (13.3%). However, molecular analysis missed one of these cases. KMT2A-r were detected in 3 patients (4%); 2 patients had t (4;11) (q21.3;q23.3) and one had an unidentified partner. Cytogenetic data were available only for 37 patients, of which 33 had abnormal karyotypes (89%). Chromosome 21 abnormalities (ch.21.abn), including translocations, gains, or loss, were the most frequently encountered (n=15).

The BCR::ABL1 fusion gene was detected more frequently in adults (p=0.046). Otherwise, there was no significant difference between BCR::ABL1 positive and negative cases in sex or different clinical characteristics (Table 2). Chromosome 21 abnormalities had no significant relation with age, sex, BM cellularity, or immunophenotypes (Table S1) but were associated with hyperdiploidy (p=0.005).

Myeloid protocols were used to treat 58 patients (77.3%), while the remaining 17 received lymphatic protocols. Ten patients received imatinib, and five were treated with BM transplantation (BMT). Table 3 shows the response to treatment on days 14, 28, and 42. By the end of the follow-up, 57 patients (76.0%) achieved CR. Six patients died earlier than 28 days. Although BCR::ABL1 positivity was not associated with CR, it was noted that all

Table 2. Response to Treatment on Days 14, 28, and 42

Parameter		Value
Day 14		
Response	Complete Remission	43 (57.3%)
	Partial Remission	5 (6.7%)
	Refractory	27 (36.0%)
Bone marrow cellularity	Hypocellular	52 (69.3%)
	Normocellular	18 (24.0%)
	Hypercellular	5 (6.7%)
Bone marrow blasts	< 5%	41 (54.7%)
	≥ 5%	34 (45.3%)
Minimal Residual Disease	< 0.1%	5 (6.7%)
	≥ 0.1%	71 (93.3%)
Day 28 (n=69)		
Bone marrow blasts	< 5%	18 (26.1%)
	≥ 5%	51 (73.9%)
Minimal Residual Disease	< 0.1%	11 (15.9%)
	≥ 0.1%	58 (84.1%)
Day 42 (n=60)		
Bone marrow blasts	< 5%	7 (11.7%)
	≥ 5%	53 (88.3%)
Minimal Residual Disease	< 0.1%	14 (23.3%)
	≥ 0.1%	46 (76.7%)
Complete Remission at the end of follow up		57 (76.0%)

Data are expressed as number (%)

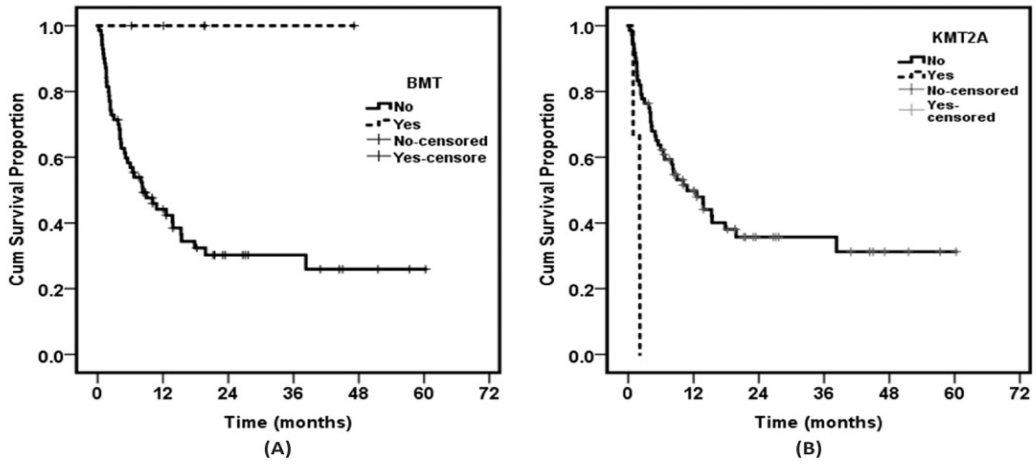

Figure 1. Overall Survival of Patients with MPAL in Relation to (A) treatment with bone marrow transplantation and (B) the presence of KMT2A rearrangement

positive BCR::ABL1 cases had MRD of 0.1% or more on day 42. Still, the relation was not statistically significant (p = 0.094) (Table 2). In addition, there was no association between ch.21.abn and response to treatment (Table S1).

The median follow-up period was 8.2 months (range: 2-60 months). Forty-six patients died during follow-up. The median overall survival (OS) was 10 months (95%CI: 4-16 months). The cumulative OS proportion at 12 months was 47.8%. The OS was not affected by BCR::ABL1 positivity or ch.21 abn (Table 4), while it was better in patients treated with BMT (p = 0.027). Although the OS was worse in KMT2A-r positive cases (p = 0.001), the conclusion may not be reliable with the low number of the cases (Figure 1). The median disease-free survival (DFS) of patients who achieved CR (n=57) was 9.4 months (95%CI: 4.4-14.3). The cumulative DFS proportion at 12 months was 42.7%. DFS was not affected by all tested prognostic factors (Table S2).

Discussion

This study shows the characteristics of 75 patients with MPAL. They were 37 children and 38 adults. The main phenotype was B/myeloid type (80%). However, according to the current WHO 2016 classification (Swerdlow et al., 2017), the present series included 13 cases of the two genomic categories. Three cases were KMT2A-r positive, and 10 were BCR::ABL1 fusion-positive. Therefore, they can be classified into four classes: BCR::ABL1 fusion-positive (n=10), KMT2A-r positive (n=3), B/myeloid NOS (n=48), and T/myeloid NOS (n=14). B/myeloid immunophenotype was also more common in previous studies in adults and children. Matutes et al., (2011) found 59%, and Yan et al., (2012) found 70% B/myeloid type in adults. Half of the children in other series had B/myeloid type (Lee et al., 2019; Chang et al., 2021).

Table 3. Demographic, Clinical Characteristics, and Response to Treatment of Patients with and without BCR::ABL1 Fusion Gene

Parameter		BCR-ABL Positive	BCR-ABL Negative	p-value
Age	Children	2 (5.4%)	35 (94.6%)	0.046
	Adults	8 (21.1%)	30 (78.9%)	
Sex	Male	8 (14.3%)	48 (85.7%)	0.677
	Female	2 (10.5%)	17 (89.5%)	
Immunophenotyping	B-lymphoid/myeloid	9 (15.0%)	51 (85.0%)	0.396
	T-lymphoid/myeloid	1 (6.7%)	14 (93.3%)	
BM cellularity	Normocellular	2 (10.5%)	17 (89.5%)	0.677
	Hypercellular	8 (14.3%)	48 (85.7%)	
Modal chromosome number	Hypodiploid	0 (0.0%)	3 (100.0%)	0.255
	Diploid	8 (36.4%)	14 (63.6%)	
	Hyperdiploid	2 (16.7%)	10 (83.3%)	
Complete Remission	Yes	8 (80.0%)	49 (75.4%)	0.75
	No	2 (20.0%)	16 (24.6%)	
MRD on day 42	< 0.1%	0 (0.0%)	14 (26.9%)	0.094
	≥ 0.1%	8 (100.0%)	38 (73.1%)	

Data are presented as number (%)

Table 4. Overall Survival of the Studied Group in Relation to Prognostic Factors

	n	n events	Cumulative survival proportion (%)	Median survival (months)	p-value
All cases	75	46	47.8	10 (4-16)	
Age group					
Children	37	23	50.2	12.6 (1.2-24.0)	0.951
Adults	38	23	45.6	10.1 (3.5-16.7)	
Sex					
Male	56	33	54.2	13.8 (6.5-21.0)	0.249
Female	19	13	28.5	6.4 (3.0-9.9)	
Immunophenotype					
B/myeloid	60	35	51.5	12.6 (6.0-19.2)	0.292
T/myeloid	15	11	33.3	5.5 (1.6-9.3)	
BCR::ABL1					
Positive	10	4	58.3	*	0.412
Negative	65	42	46.4	8.0 (2.5-15.7)	
KMT2A-r					
Yes	3	3	0	2.1 (0.2-4.0)	
No	72	43	49.8	10.8 (5.3-16.3)	0.001
Cytogenetics (n=37)					
Normal	4	3	50	6.4 (0.0-30.8)	0.684
Abnormal	33	17	50.1	15.4 (0.0-31.6)	
Ch.21 abn. (n=37)					
Yes	15	6	64.6	*	
No	22	14	40	4.1 (0.0-10.5)	0.131
CTH					
Myeloid	58	38	46.3	10.1 (3.7-16.5)	0.131
Lymphatic	17	8	52.9	*	
Imatinib					
Yes	11	5	51.1	*	
No	64	41	47.1	10.1 (2.5-17.7)	0.36
BMT					
Yes	5	0	100	*	
No	70	46	44.2	*	0.027

*, Median survival cannot be calculated; BMT, Bone marrow transplantation

It is assumed that MPAL evolves from multipotent stem cells, capable of differentiating into myeloid and lymphoid lineages during the development of acute leukemia (Lee et al., 2019). It has been shown that CD34 is primarily considered a marker of hematopoietic stem cells and hematopoietic progenitor cells (Sidney et al., 2014). We found CD34 in 82.7% of patients, and this finding supports the view that MPAL cells originate at the early stages of hematopoietic differentiation.

Among 37 patients with available karyotype, only four had a normal karyotype, and four had a complex one defined as ≥3 structural abnormalities. Therefore, about 11% of our patients had complex karyotypes. These figures are lower than those reported by Matutes et al., (2011) and Yan et al., (2012), who found 32% and 24% complex karyotypes in their series, respectively. This discrepancy could be explained by the different used techniques to detect the fusion genes and other cytogenetic aberrations and due to ethnic variations; Matutes et al., (2011) performed FISH analysis for the screening of BCR::ABL1, ETV6::RUNX1, and KMT2A rearrangements while in our study only BCR::ABL1 fusion gene was screened by FISH. The higher incidence of complex karyotypes reported by Yan et al., (2012) is due to the fact of using multiplex reverse transcription-polymerase chain reaction (RT-PCR) to detect 29 acute leukemia-related fusion genes in adult Chinese patients which may explain the higher incidence of reporting abnormal cytogenetic results and subsequently complex karyotype. In addition, the cohort assessed in the mentioned study included adult patients only while our cohort included pediatric and adult patients with MPAL. On the other hand, Weinberg et al., (2014) found 44% of their series with normal karyotypes.

MPAL has no specific chromosomal abnormalities. Owaidah et al. (2006) reported clonal abnormalities in 68% of MPAL patients; KMT2A-r was the most common, followed by BCR::ABL1 (Alexander et al., 2018). In agreement with Chang et al., (2021), clonal cytogenetic

abnormalities were found in 89% of MPAL patients in the present series. The BCR::ABL1 fusion gene was detected in 8 adults compared to two children (p=0.046). Besides, all KMT2A-r positive cases were adults. Previous studies have shown that BCR::ABL1 translocation is uncommon in children, while 15% of MPAL cases in children harbor KMT2A-r (Alexander et al., 2018). Lee et al., (2019) detected one BCR::ABL1 positive patient, and two had KMT2A-r. In another study, BCR::ABL1 and KMT2A-r were mainly found in B/myeloid MPAL (Cao et al., 2019).

However, the most frequent genetic abnormality in this series was ch.21.abn found in 15 patients (20%), mainly in B/myeloid cases (n=11). Previous studies reported different frequencies of ch.21 abn ranging from 8% to 26%. In contrast, Chang et al., (2021) reported that 50% of pediatric MPAL patients were associated with ch. 21 abn. However, most of these studies had a small sample size (Yan et al., 2012; Takahashi et al., 2018; Quesada et al., 2018; Eckstein et al., 2016). Chromosome 21 abnormalities were detected in patients with B/myeloid phenotype (Cao et al., 2019). In a sample of 23 adult and pediatric MPAL patients, ch.21 abn were found in 2 patients: one child and one adult (Eckstein et al., 2016). The largest series of Alexander et al., (2018) found 11% ch.21 abn. Thus, the present study detected the highest frequency of ch.21.abn with a relatively large sample size.

Despite its rarity, MPAL has a poor prognosis compared to other subtypes of acute leukemia. Therefore, proper diagnosis of MPAL is essential for a successful outcome in these patients (Porwit and Béné, 2019). Precise diagnosis necessitates a careful assessment of the clinical, immunophenotypic, and genetic data (Khan et al., 2018). We defined the disease according to the WHO criteria in the current series. The WHO criteria used fewer parameters than the European Group for the Immunological Classification of Leukemia (EGIL) scoring system (Weinberg et al., 2014); however, it used measures from cytometry and cytochemistry (Lee et al., 2019). The WHO 2016 classification defined three categories: associated with BCR::ABL1 fusion gene, associated with KMT2A rearrangements, and non-otherwise specified (Béné and Porwit, 2012).

At present, there is no consensus about the treatment of MPAL. It is controversial to use single chemotherapy or combined therapy for lymphoid and myeloid leukemia (Zheng et al., 2021). A systematic review investigated the efficacy of different regimens on disease response and survival. The study included data from 97 reports, including 1351 patients. The meta-analysis showed that AML induction was less likely to attain a CR than ALL regimens. Also, OS was better for patients beginning with ALL versus AML therapy (Maruffi et al., 2018). Cellular and molecular genetic abnormalities can help for guiding the proper treatment strategy of MPAL patients (Matutes et al., 2011; Yan et al., 2012). Nevertheless, lacking robust prospective trials is the main obstacle to reaching a standard approach for treating MPAL as a heterogeneous disease.

In summary, this study demonstrates that MPAL is a complex entity with heterogeneous clinical, immunophenotypic and genetic characteristics. The main immunophenotype was B/myeloid type, and BCR::ABL1 and ch.21 abn are the most frequent associated abnormalities. The treatment outcome is generally poor, and patients subjected to BMT had better overall survival. Accurate diagnostic criteria are essential for tailoring specific treatment strategies. Other trials are needed to standardize the optimal therapeutic modality of MPAL cases.

Author Contribution Statement

All authors have contributed to the manuscript in significant ways have reviewed and agreed upon the manuscript content. Eman Z. Kandeel: Conceptualization, Methodology and Validation; Naglaa M. Hassan: Investigation, data curation and reviewing; Mona S. El Ashry: Investigation, Data curation, Writing- Original draft preparation.

Acknowledgements

The authors would like to thank all the patients who were included in this study.

Declarations

Ethics approval and consent to participate: Every patient gave a written informed consent. The study was conducted in accordance with the Helsinki declaration of 2011 and was approved by the National Cancer Institute's internal review board.

Consent for publication

Not applicable.

Availability of data and material

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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