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CASE REPORT

Mixed phenotype acute leukaemia with t(9,22), BCR-ABL1: A case report

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Abstract

Introduction: Mixed phenotype acute leukaemia (MPAL) is a rare entity of acute leukaemia. *Case Report:* Here we report a case of a 39-year-old lady, with an incidental finding of hyperleukocytosis (white blood cells count: 139.2 x 10^9/L). Her peripheral blood film revealed 36% of blasts and a bone marrow aspiration showed 53% of blasts. Immunophenotyping showed a population of blasts exhibiting positivity of two lineages, myeloid lineage and B-lymphoid lineage with strong positivity of CD34 and terminal deoxynucleotidyl transferase (Tdt). A conventional karyotyping revealed the presence of Philadelphia chromosome. She was diagnosed with MPAL with t(9,22), BCR ABL1, which carried a poor prognosis. She was treated with acute lymphoblastic leukaemia (ALL) chemotherapy protocol coupled with a tyrosine kinase inhibitor and was planned for an allogeneic stem cells transplant. *Conclusion:* This MPAL case was diagnosed incidentally in an asymptomatic patient during medical check-up. We highlight this rare case report to raise the awareness about this rare disease. Understanding the pathogenesis of the disease with the underlying genes responsible for triggering the disease, uniform protocols for diagnosis and targeted treatment will help for proper management of these patients.

Keywords: Mixed phenotype acute leukaemia, Philadelphia chromosome, ALL chemotherapy

INTRODUCTION

Acute leukaemia is classified as either acute lymphoblastic leukaemia (ALL) or acute myeloid leukaemia (AML), based on the morphology, cytochemical staining, immunophenotypic profiles and genetic profiles.[1] However, there is a minority of acute leukaemia which has no specific lineage or exhibits antigens expression of more than one lineage. This heterogeneous group of leukaemia with markers of multiple lineages is currently referred as mixed phenotypic acute leukaemia (MPAL).[2] Historically, MPAL had undergone several changes over a period of time in term of its nomenclature and the diagnostic criteria. However, according to the new revised 2016 World Health Organization haematological classification, it is classified under acute leukaemia of ambiguous lineages.[3,4] Five subtypes of MPAL are defined: MPAL with t(9;22)(q34;q11.2), MPAL with MLL rearrangement, MPAL B/myeloid not otherwise specified (NOS), MPAL T/myeloid NOS, and MPAL NOS rare types.[4] These MPAL cases are subdivided into bilineal and biphenotypic depending on the presence of blast population and expression of markers. In bilineal MPAL, two morphologically and immunophenotypically distinct populations of blasts are present while in biphenotypic MPAL, there is one blast cell population expressing markers of more than one lineage.[5]

Mixed phenotypic acute leukaemia accounts for 2-5% of all acute leukaemia and is considered as a disease with poor clinical outcome.[1,4,6] It is a rare disease and there is limited knowledge or studies on the disease. Due to the rarity of the disease, knowledge of its pathogenesis, disease progression and treatment is limited to some retrospective analyses of small patient cohorts. There is no clearly defined standard therapy

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for MPAL, which often leads to inconsistency in treatment options between AML-directed regimens and ALL-directed regimens. Stem cell transplantation is recommended in patients responded with chemotherapy.[2] The underlying molecular pathophysiology is still not clearly defined. Better understanding of the molecular landscape can help to select the better treatment choices.[7] We report here a case of MPAL with t(9;22), BCR ABL1, incidentally identified in an asymptomatic adult women during her medical check-up.

CASE REPORT

A 39-year-old lady, with no known medical illness, was incidentally noted with hyperleucocytosis during a pre-hajj medical check-up. Clinically she was well, without any constitutional symptoms such as fever, weight loss or loss of appetite. Physical examination was unremarkable. There was no hepatosplenomegaly or lymphadenopathy. A full blood count showed mild anaemia (haemoglobin: 10.9g/dL) with high white blood cells count (139.2 x 10^9/L), predominantly neutrophils (73.7 10^9/L) and followed by lymphocytes (39.3 x10^9/L). Platelets count was within normal range (154 x 10^9/L).

A full blood picture revealed 36% of blasts, which appeared moderate to large in size, with scanty cytoplasm, fine chromatin pattern and some blasts with prominent nucleoli. There was background of neutrophilia and lymphocytosis. However, there was no basophilia, eosinophilia or dysplastic features seen (Fig. 1). A bone marrow aspiration revealed hypercellular fragments and hypercellular cell trails with 53% of blasts. There was lymphocytosis (21%) with relatively reduction of erythroid and granulocytic series (Fig. 2). A cytochemical staining showed myeloperoxidase was positive for the blasts population.

Immunophenotyping analysis was performed on the bone marrow aspiration (Fig. 4). CD45 vs side scatter (SSC) strategy was used to gate the blast population. A single population of blasts was gated, which account for 30% of the total cells population. The blasts exhibited two lineages, which were the myeloid and B-lymphoid lineages. They showed positivity for myeloperoxidase, CD33, CD13, CD19, CD79a, CD10 and CD22. Stem cells markers (CD34) and terminal deoxynucleotidyl transferase (Tdt) were strongly positive. The blasts were negative for T-lymphoid cells markers and monocytic cells markers.

Trephine biopsy (Fig. 3) supported the diagnosis showing diffuse infiltration of blasts with positivity in myeloperoxidase, CD79a, CD34 and Tdt. A conventional karyotyping (Fig. 5) showed translocation of the long arm of chromosome 9 and chromosome 22, which was the presence of Philadelphia chromosome.

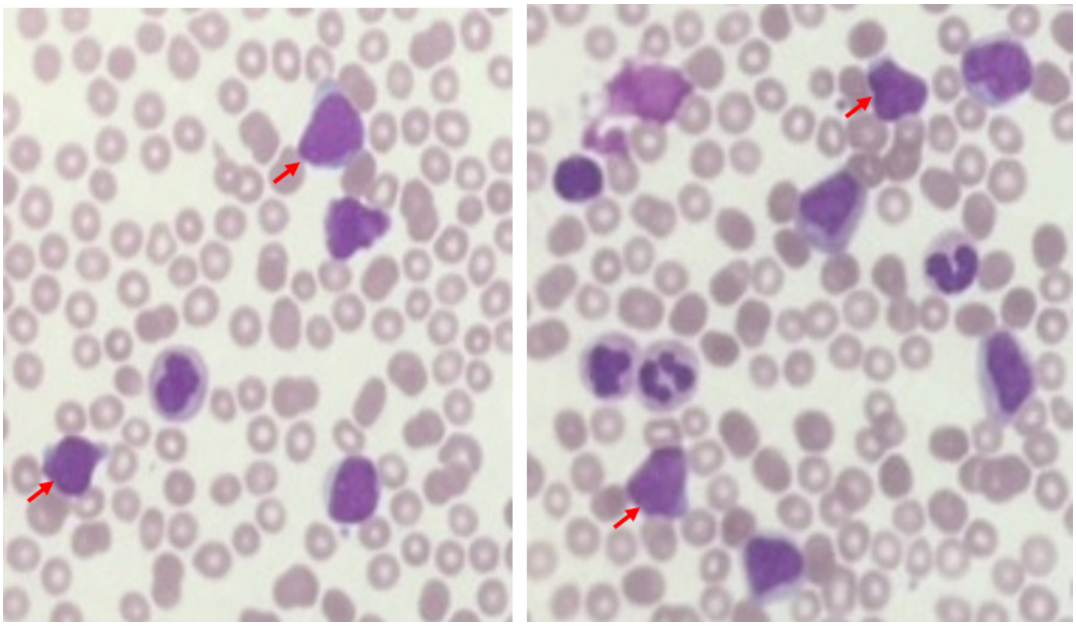

FIG. 1: Peripheral blood film under light microscope (x40) showing blasts (red arrows), which appear moderate to large in size, with scanty cytoplasm, open chromatin pattern and some with prominent nucleoli.

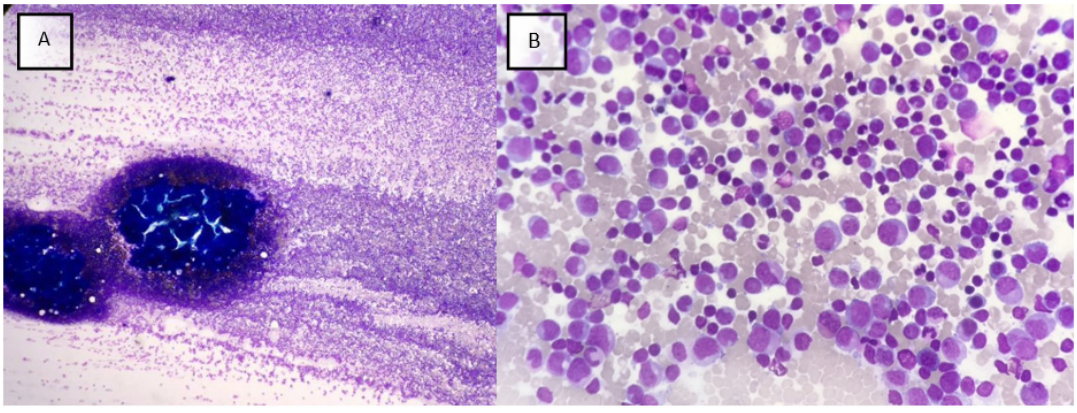

FIG. 2: Bone marrow aspiration under light microscope (A) (x10) showing hypercellular fragments and hypercellular cells trails. (B) (x20) showing presence of blasts with background of lymphocytosis with relatively reduced erythroid cells and granulocytic cells.

She was then diagnosed as a distinctive entity of mixed phenotypic acute leukaemia with t(9,22).

She was treated with alternate course A and course B of HyperCVAD/methotrexate-cytarabine chemotherapy regime. For course A, she was given intravenous (IV) cyclophosphamide 300 mg/m^2 for 12 hour at day 1 to day 3, IV doxorubicin 50 mg/m^2 at day 4 and day 5, IV infusion of vincristine 1.4 mg/m^2 at day 4 and day 11, and oral dexamethasone 40mg at day 1 to 4 and day 11 to 14. For course B, she was given IV methotrexate 1 g/m^2 for 24 hours continuously, with 200 mg/m^2 for first two hours, then 800 mg/m^2 for the next 22 hours at days 1–2, and IV cytarabine 3g/m^2 over two hours, 12 hourly at day 2 and day 3. She was also given daily tyrosine kinase inhibitor (Imatinib). She was planned for allogenic stem cell transplant once first complete remission was achieved.

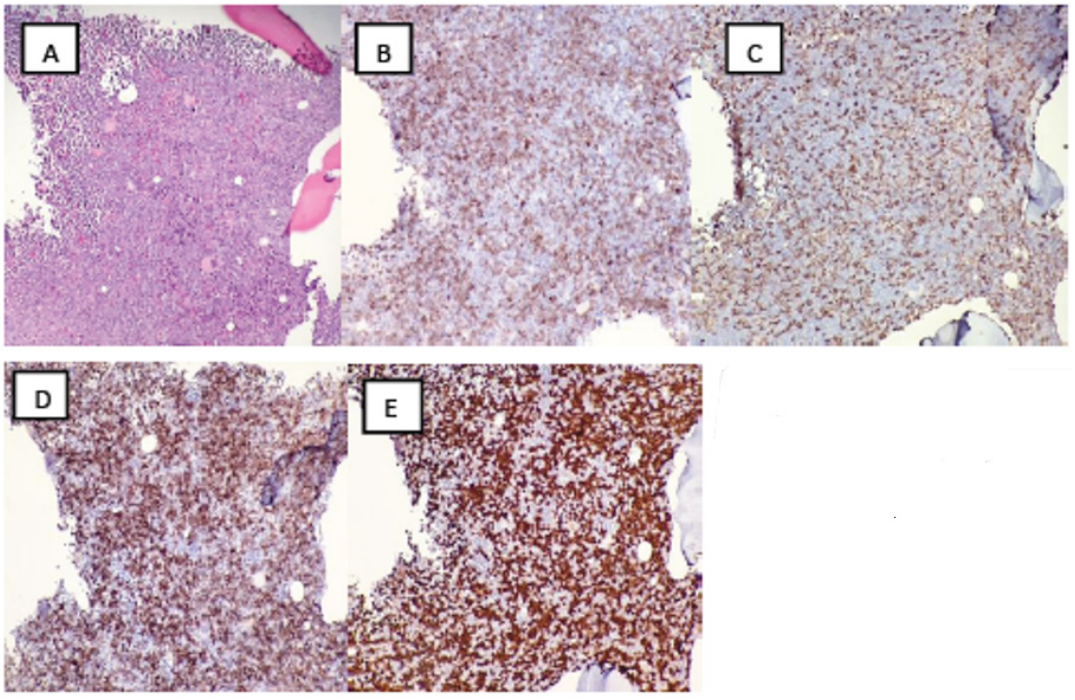

FIG. 3: Trephine biopsy under light microscopy (X20) showing diffuse infiltration of blasts (A) H&E, (B) positive CD79A, (C) positive myeloperoxidase, (D) positive CD34, and (E) positive Tdt.

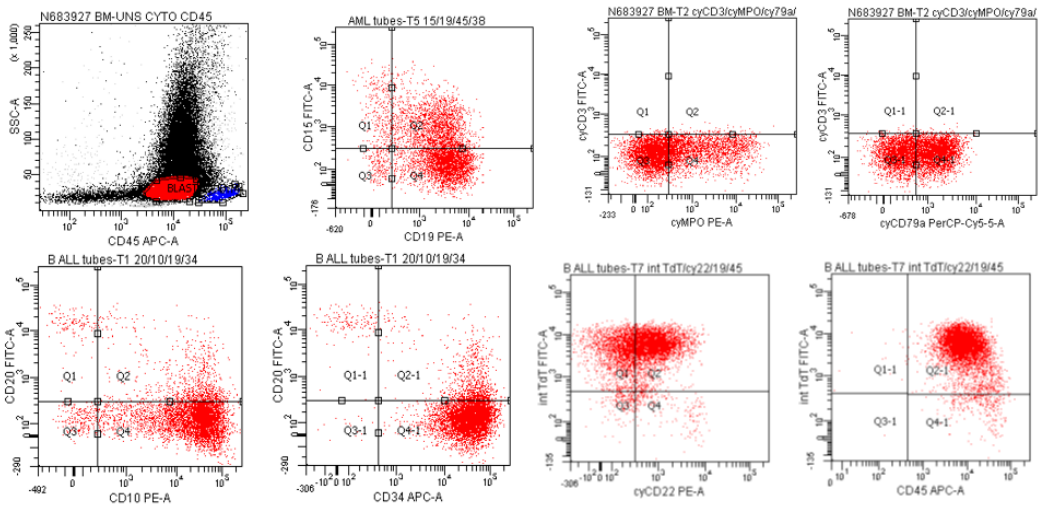

FIG. 4: Immunophenotyping of the bone marrow aspiration showed blasts were gated at blasts window on CD45 vs SSC-A. The blasts showed positivity in CD19, cyMPO, cyCD79a, CD10, HLA-DR, CD34 and Tdt.

DISCUSSION

Patients with MPAL typically present with symptoms of an acute leukaemia such as anaemia, recurrent infection or bleeding tendencies. In addition, hepatosplenomegaly or lymphadenopathy can also be present.[1,8] However, in this present case report, our patient was asymptomatic and was diagnosed with acute leukaemia after an incidentally findings of hyperleucocytosis during the pre-Hajj medical check-up. Her condition can be hypothesised as her haemoglobin level was just slightly reduced which does not pose her any anaemic symptoms. Her neutrophils count and platelet count were still within normal and functional limit at diagnosis. This hindered her from any recurrent infection or bleeding event. Though she was asymptomatic, the finding of hyperleucocytosis warranted a further workout for any underlying haematological disorder and discovered this rare malignancy.

According to 2016 WHO classification, the diagnosis of MPAL is based on the presence of specific cytochemical and immunophenotypic markers which are characteristic of the developmental lineages.[2,3,4] The blasts population has to be more than 20 per cent with the presence

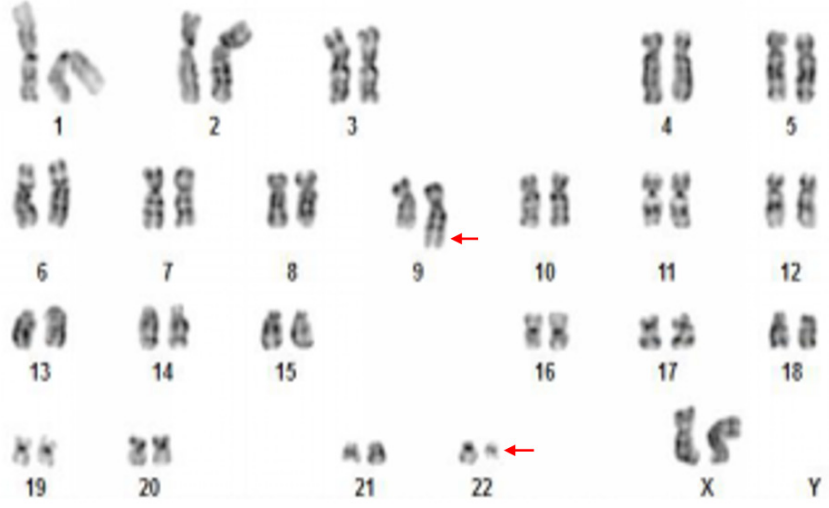

FIG. 5: A conventional karyotyping showed a translocation of the long arm of chromosome 9 and chromosome 22 (red arrows).

of antigenic expression of more than one lineage.[2] Myeloid lineage is determined by the presence of myeloperoxidase (MPO) positivity on the leukaemic cells. The MPO positivity should be demonstrated on an aberrant population in flow cytometry or appears more than 3% of blasts in a cytochemical study. Monocytic differentiation is determined by the presence of at least 2 markers as following: non-specific esterase, CD64, CD14, CD11c or lysozyme. T cells lineage is defined with the presence of surface/cytoplasmic CD3. For B-cells lineage, a strongly expressed CD19 and the presence of at least one of the other B cells markers (CD10/cyCD22/CD79a) are required. With a weak CD19 expression, the presence of at least two other B cells markers are needed.[3] Besides expressing antigenic profile of multiple lineages, the blasts commonly express early haemopoietic stem cells markers such as CD34 or terminal deoxynucleotidyl transferase (Tdt).[2] Thus, it is postulated that the blasts in MPAL are derived from very early haemopoietic progenitors which harbour the potential to be subjected to either myeloid or lymphoid differentiation.[1,2,9] An earlier study showed that among 100 patients with MPAL, there was strong expression of Tdt in 89% of the cases and CD34 in 74% of the cases.[9]

Due to co-expression of antigenic markers from different lineages, MPAL is classified as B/myeloid leukaemia, T/myeloid leukaemia, T/B lymphoblastic leukaemia or rarely trilineage leukaemia.[3] The B/myeloid leukaemia is being the most common subtype.[10-12] There are two distinctive subgroup of MPAL which are further classified based on its genetic abnormalities, which are the MPAL with the presence of the Philadelphia chromosome or *BCR/ABL1* rearrangement, and MPAL with rearrangement of the mixed-lineage leukaemia (*MLL*) gene.[2,3] Herein, our patient's immunophenotypic profile and immunochemical staining results confirm the diagnosis of MPAL with B/myeloid leukaemia subclass, with a special entity of Philadelphia chromosome (BCR-ABL1).

However, there are some challenges in the diagnosis of MPAL. A small number of acute myeloid leukaemia (AML) which harbours translocation t(8,21), a type of AML with good prognostic outcome, can express multiple B cells markers. Nevertheless, they are not considered as bi-phenotypic.[2] Furthermore, chronic myeloid leukaemia in blasts crisis can also express a variety of lineages.[2] The later one is similar to MPAL with Philadelphia chromosome and poses challenges to differentiate one from the other. In this present case report, our patient presented without a splenomegaly, and her peripheral blood smear did not show the background of chronic myeloid leukaemia, she was thus diagnosed as MPAL with Philadelphia chromosome instead of chronic myeloid leukaemia in blast crisis. It is stated that, Philadelphia chromosome with occurrence rates around 20% to 40% represents the most common cytogenetic abnormality in MPAL cases.[12]

There are a few current studies performed on the genetic landscape to detect the frequencies and types of genetic mutations of MPAL. In 2016, Eckstein *et al*. studied on 23 MPAL patients aged 2–74 years using Whole Exome Sequencing (WES) showed, 8/23 samples (35%) had mutations in epigenetic regulatory genes where DNMT3A was the most frequently mutated gene in adults (33%). Other mutations in epigenetic regulatory genes were found in IDH2 (9%), TET3 (4%), and EZH2 (9%). It is mentioned that MPAL patients with a mutation in DNMT3A occur mostly in adult patients. Mutations of activated signalling pathways (NRAS, KRAS, NF1, and FLT3), tumour suppressors (TP53, WT1) and transcription factors (NOTCH1, RUNX1 and GATA2) were also frequent. This study suggest that these mutations arise early in disease development.[13] High mutation frequency for DNMT3A (10/18 patients; 55.6%) in adults with T-myeloid MPAL was also supported by Kern *et al*. in 2012.[14] More recent study by Queseda *et al*. *in* 2018 using next-generation sequencing (NGS) panel showed that almost two thirds of cases (9/14, 64%) of MPAL detects 25 distinct mutations involving 14 gene namely ABL1, ASXL1, DNMT3A, EGFR, FLT3, GATA1, IDH1, IDH2, JAK2, NOTCH1, NRAS, RUNX1, TET2, TP53 and WT1. Internal tandem duplications in FLT3 (FLT3-ITD) were the only recurrent mutation in 2 patients. In 2/7 cases of B/My MPAL patients had t(9;22)/BCR-ABL1 rearrangement that did not show any mutations. Two patients with KMT2A rearrangement had mutations. They found B/My MPAL cases had less mutations compared with T/My MPAL cases (43% vs. 100%). In contrast, B/My MPALs more commonly showed a complex karyotype compared to T/My MPALs (71% vs. 17%). With NGS and karyotype combined, most (93%) MPAL cases had mutations or cytogenetic abnormalities.[5] A study by Takahashi *et al*. in 2018 showed that the most frequently mutated genes were NOTCH1in 29% cases

(all myeloid-T), RUNX1 in 26% cases (six myeloid-B and two myeloid-T), and DNMT3A and IDH2 in 23% cases each (one myeloid-B and six myeloid-T for both). Their findings showed that around 50% of MPAL patients carried at least one clinically actionable mutation (IDH2 and FLT3).[7] A study on the paediatric cases by Alexander *et al*. in 2018 on 115 patients with MPAL using whole-genome, whole-exome, and RNA sequencing has shown that commonly mutated genes identified are similar to that identified in AML are: FLT3, RUNX1, CUX1 and CEBPA, similar in ALL are: CDKN2A or CDKN2B, ETV6, and VPREB1 and similar in both AML and ALL are WT1 and KMT2A. They revealed that the two most common subtypes of MPAL are B/myeloid and T/myeloid. The important finding they showed that 48% of B/myeloid MPAL cases carried rearrangements in ZNF384, a characteristic similar to B-cell ALL. The researchers mentioned that the gene expression profiles of ZNF384r B-ALL and ZNF384r MPAL are indistinguishable. They noted that alterations in signalling pathway genes are common in all subsets of MPAL with mutations in FLT3 being the most common signalling mutation and occurring in all subsets, but more frequently in T/M MPAL. Thus, patients with ZNF384r exhibited higher FLT3 expression than patients with other types of B/myeloid or T/myeloid MPAL. This study identifies that mutations occur earlier in cell development that retain the potential to acquire myeloid or lymphoid features.[15] Studies comparing the adult and paediatric age group showed, adult MPAL patients revealed mutations in genes mostly associated with DNMT3A[13,14] while ZNF384 rearrangements identified in the paediatric cohort.[15]

There is no established protocol for treatment of MPAL and different therapeutic approaches have been reported. The published data suggests that there is better outcome with ALL chemotherapy protocol followed by allogeneic stem cell transplant.[9,2] Previous study on 11 children with MPAL, treated with ALL-like induction therapy showed complete remission was achieved by all children.[16] Another study showed that ALL chemotherapy protocol is superior to AML chemotherapy protocol. It was evidenced by 23 out of 27 patients (85%) who had achieved complete remission after using ALL chemotherapy regime, while only 14 out of 34 patients (41%) attained complete remission after receiving AML chemotherapy protocol.[9] Another study also proved that 8 out of 10 patients who failed to respond to AML therapy attained a complete remission after receiving ALL induction therapy.[17] Apart from that, studies have shown that patients who undergo allogeneic stem cells transplants have a better overall survival.[2,11] In comparison to patients who received only consolidation chemotherapy, they have a longer overall survival (22 vs 9 months).[11] As it is postulated that MPAL carries blasts that are derived from the primitive long-lived haemopoietic stem cells which are capable of lineage infidelity, chemotherapy alone would be insufficient to eradicate the disease. Thus, allogeneic stem cells transplant is recommended in most of the studies.[2,11]

A study on molecular pathophysiology identifying mutation shown the genetic and molecular markers to be considered before initiating treatment as some of the mutations such as IDH1/2, BRAF, NOTCH1, FLT3 could be therapeutically targetable with currently available agents or in various stages of clinical development including epigenetically targeted agents, such as tyrosine kinase pathway inhibitors, and NOTCH1 inhibitors.[13] There are new drugs recently approved by FDA such as midostaurin (an oral FMS-like tyrosine kinase 3 (FLT3) inhibitor) and enasidenib (an oral Isocitrate dehydrogenase-2 (IDH-2) inhibitor) which are clinically effective in patients with FLT3 and IDH2 mutations respectively.[7,18] Paediatric patients with ZNF384r mutation with higher FLT3 expression also might respond well to treatment with a FLT3 inhibitor.[15]

A few studies showed that patients with MPAL with Philadelphia chromosome demonstrated adverse prognosis, with a median survival of 8 months in comparison to 139 months in MPAL with normal karyotype.[3,9] Tyrosine kinase inhibitors (TKIs) targeting the BCR-ABL1 protein has significantly altered the treatment protocol and improved the prognosis of Philadelphia positive leukaemia.[19] In MPAL, presence of Philadelphia chromosome demands a specific approach by using tyrosine kinase inhibitor in combination with the ALL chemotherapy regime, which is then consolidated with allogeneic stem cell transplant if feasible.[2] In this present case, our patient is also given the Tyrosine kinase inhibitor with other chemotherapy regime and planned for allogeneic stem cells transplant once she has achieved complete remission.

The survival rates for children with MPAL is 47–75%, while in adults with MPAL are

20–40%.[15] The poor prognosis of MPAL compared to other types of leukaemia are due to i) the mixed-phenotype leukaemic stem cells are chemoresistant owing to slow replication, ii) the blasts can adapt to therapy by switching phenotype, and iii) some MPALs express high levels of multidrug resistance proteins.[20] Nevertheless some other factors may also affect the outcome of the disease such as the age of the patient, any additional undiscovered molecular abnormalities, failure to response to induction therapy and the type of post remission therapy.[2] However, based on the few larger retrospective studies with WHO defined MPAL, complete remission rate is reported at 61.5-85.2% and median OS is at 14.8-18 months.[2]

CONCLUSION

As MPAL is a rare leukaemia and there is limited information about the disease, efforts need to be made worldwide in order to establish the natural history and behaviour of this disease, as well as to discover the putative deregulated genes responsible for triggering the disease. Thus, with the understanding of the pathogenesis of the disease, uniform protocols for diagnosis and targeted treatment of these patients should be devised and followed.

Authors' contribution: All authors contributed equally to the study conception and design. WLY collected the case report history and initial drafting the manuscript. SAA and RY contributed in final drafting of the manuscript. NY, AI, SS, RT and SAA contributed in critical review of the manuscript. Finally, all authors have read and approved the manuscript.

Conflict of interest: The authors declare no conflict of interests.

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