A RARE CASE OF MIXED LINEAGE LEUKEMIA IN AN IDENTICAL TWINS: A CASE REPORT

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ABSTRACT

Leukemia is the most malignant neoplasm in childhood, accounting for approximately 31% of all malignancies that occur in children younger than 15 years. Leukemia in infants is rare and reaches approximately 2% cases of leukemia during childhood before age 1 year. However, this disease generates tremendous interest due to its aggressive clinical presentation in a uniquely vulnerable host, its poor response to current therapies, and its unique biology that is increasingly pointing the way toward novel therapeutic approaches. We were herein to report a rare case of leukemia in identical twins involving mixed-lineage leukemia (MLL). Identical twins, boy-boy twins, delivered by C-section to a 38-year-old G2P3 at 34 weeks' gestation, the weight of child no.1 and child no.2 at birth was 2300 gram and 1800 gram, relatively. Child no.1 was admitted to the hospital with fever and cough at 1 month 8 days old. He had been coughing and having a fever for 2 days. He was diagnosed with a case of upper respiratory tract infection; thus a complete blood count (CBC) was indicated to assist. The result of white blood cell count was much higher than the normal value, so he was suspected to be a case of acute leukemia. He was brought to Blood Transfusion Hematology Hospital where he was diagnosed as a case of mixed-lineage leukemia based on peripheral blood finding, flow cytometry, fluorescence in situ hybridization (FISH), and karyotype test. At the same time, child no.2 was brought in by his mother for screening tests, and the result of peripheral blood findings was normal. When child no.2 at 1 month 25 days old, he was admitted to the hospital after 2 days of fever, coughing, and wheezing. He was diagnosed with severe pneumonia/acute leukemia based on peripheral blood findings.

Keywords: *Flow cytometry; identical twins; karyotype; keukemia.*

I. INTRODUCTION

Leukemia is the most common malignant neoplasm in childhood, accounting for approximately 31% of all malignancies that occur in children younger than 15 years old. Each year, leukemia is diagnosed in approximately 3,100 children and adolescents <20 years old in the United States, an annual incidence of 4.5 cases per 100,000 children [4]. Acute leukemias illustrate a clonal expansion and arrest at a specific stage of normal myeloid or lymphoid hematopoiesis. They generate 97% of all childhood leukemias and are involved of the following types:

• Acute lymphoblastic leukemia (ALL)—75%: Among ALL, the nomenclature of the subtypes has been changed from "precursor B-lymphoblastic leukemia/lymphoma" and "precursor T-lymphoblastic leukemia/lymphoma" to "B or T-lymphoblastic leukemia/lymphoma" respectively.

• Acute myeloblastic leukemia (AML), also known as acute non-lymphocytic leukemia—20%.

• Acute undifferentiated leukemia—0.5%.

• Acute mixed-lineage leukemia [15].

In the first year of life in the United States, the incidence of acute leukemia is 30 cases per million live births. The annual incidence of ALL (20 per million) is almost twice the rate of AML (10.6 per million) [8]. While 2.5% to 5% of pediatric ALL occurs in infants, AML in infants comprises 6% to 14% of pediatric AML [13]. In infants and young children *MLL* gene translocations have in up to 80% of cases of ALL, in up to 50% of cases of AML in infants, and in 80% of monoblastic variants of AML [1],[2],[9],[11]. Precursor B-cell ALL is more common than T-cell disease, accounting for approximately 80% of cases of ALL. AML accounts for 18% of cases of childhood leukemia, and CML is very rare and tends to occur in adolescents. Monozygotic twins have a 10% to 15% similarity rate for ALL [7]. The rate of twin births in the United States is 24.6 per thousand live-born infants, the annual incidence of leukemia in infants is 30 per million [11] and the concordance rates in identical twins are between 5% to 25% [3]. Leukemia in infant twins is relatively rare generally diagnosed at the same ages [3],[6],[10]. *MLL* gene translocations are approximately two-thirds by standard cytogenetic techniques; the remainder is detected only by molecular analysis or by fluorescence in situ hybridization (FISH) analysis using *MLL*-specific probes [11].

While ALL patients *MLL* gene rearrangements are most frequent in infants (80% of cases), that of older children reaches a small percentage (< 2%). Application of split-signal FISH as a screening for *MLL* gene rearrangements revealed an unprecedentedly high incidence of these aberrations in childhood AML. Molecular analysis of *MLL* gene fusions and breakpoints shows several different mechanisms leading to these chromosome aberrations [14].

Identical infant twins with concordant leukemia were first described in 1882, since that time (then) many pairs of infants and older children have been described. It has been recognized that this situation offers a unique opportunity to identify aspects of the developmental timing, natural history, and molecular genetics of pediatric leukemia in general. This anecdote was followed considerably later by a few case reports beginning in the 1930s and subsequent reviews on the topic in the 1960s and 1970s. All told, more than 70 same-sex or known monozygotic twin pairs with the concordant disease have been

recorded in variable detail. Concordant leukemia in unlike sex or known dizygotic twin pairs is exceedingly rare [12].

The most common chromosome translocation in infant leukemia results in the fusion of the MLL gene at 11q23 with a variety of partner genes, but principally AF4 in ALL. As an example, for this, in 1993, Ford et al [5] showed that, in 3 pairs of identical twin infants with concordant ALL, each pair shared the same MLL gene rearrangements as indicated by identical-sized restriction fragments (in Southern blots) of MLL with multiple enzyme digests.

II. MATERIALS AND METHODS

We presented a case of identical twins who had severe pneumonia, hemorrhage, hepatosplenomegaly, palpable lymph nodes, abnormal complete blood count and peripheral blood smear with highly increased white blood cells, a large amount of lymphoblast, reduced red blood cells and platelets.

III. RESULTS

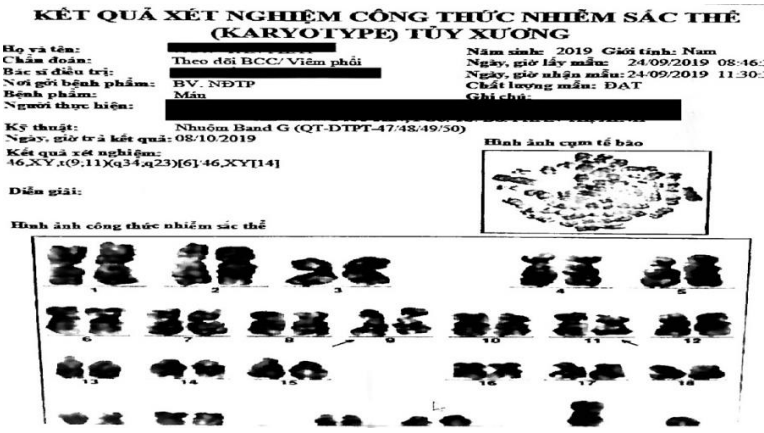
Child no.1

A 1-month-8-day-old boy patient from Binh Thuy, Can Tho, Vietnam was admitted to the Can Tho Pediatrics Hospital because his mother observed his symptoms with fever and coughing for about 2 days. Clinicians diagnosed him with an upper respiratory tract infection and indicated a complete blood count to confirm. However, the number of the white blood cells was too high, demonstrating clinicians suspect him as a case of acute leukemia. Therefore, he was referenced to Blood Transfusion Hematology Hospital for further tests including peripheral blood finding, flow cytometry, fluorescence in situ hybridization (FISH), and karyotype test. The result confirmed he had mixed lineage leukemia.

Two weeks before this time admission, his mother realized he had fever and cough as well as the appearance of wheezing for about 2 days. In Can Tho Pediatrics Hospital, he was diagnosed with severe pneumonia/acute leukemia and was admitted to ICU. In a bad (hazardous) condition that his endotracheal intubation and mechanical ventilation to support him through breathing failures, by the way, he also received medicine through I.V catheters since his infection was critical. During 10 days in ICU, he continued to have a fever, recurrent vomiting, constipation, sometimes he passed black tarry stool while other times he excreted feces with fresh red blood mixed in. His family understood their son’s situation was not bright and intensive therapy didn’t (failed to) be effective so (hence) they signed to take their child home and prepared themselves for the worst case. However, he still could weakly breathe for the next day, continued to appear the symptoms with fever, coughing, and wheezing. Therefore, he was taken back to the hospital and admitted to Hematology Department as his family’s wish.

Physical examination was remarkable. He was lethargic with recurrent febrile episodes. Signs of severe pneumonia involved oxygen saturation of 70% by pulse oximetry, shortness of breath, fast respiratory rate of 50 breaths/min, contractions of the intercostal spaces, coarse breaths sound in all lung fields. He had petechiae on the skin, purpura at the injection site, and red blood stool. Abdominal examination showed abdominal distention, collateral circulation, hepatosplenomegaly. Inguinal lymph nodes and axillary lymph nodes were palpable.

Complete blood count illustrated that white blood cells were 651.900/mm^3 with 40,7% of lymphocytes and 2% of neutrophils; hemoglobin was 7 g/dL, normocytic normochromic red blood cells, and hematocrit was 16,8%; platelets were 9.000/mm^3. Peripheral blood findings showed B-ALL type leukemia with blast cell 80%. Leukemia-associated immunophenotypes (LAIPs): CD45^{Inter}, CD34$^{\pm}$ TdT$^{\pm}$ (20%), CD19^{+} CD22^{+} CD38^{+} CD58^{+} CD81^{+} CD9^{+}, CD123$^{\pm}$ (50%), CD15$^{\pm}$ (24%), HLA-DR^{+} cyCD79a^{+}, CD10^{-} CD20^{-} CD3^{-} CD7^{-} CD33^{-} CD13^{-} CD117^{-} CD66c^{-}. The fluorescence in situ hybridization (FISH) test was usually used to diagnose many types of chromosomal abnormalities in patients with leukemia. The result of FISH in this boy (Probe: LSI MLL Dual Color, Break Apart Rearrangement Probe – 11q23 (catalog 08L57-020)) showed 96% of 200 cells taken for investigating had rearrangement of 11q23 chromosome which means there was a mutation at q23 location on autosome 11 containing MLL gene. Karyotype was another test specific for leukemia to identify and evaluate the size, shape, and number of chromosomes in a sample of body cells. In this patient, the chromosome set was 46, XY, t(9;11)(q34,q23)[6]/46,XY[14] (**Figure 1**). The best explanation for this Karyotype was that there was a balanced translocation between autosomes 9 and 11 at q23 area (t(9;11)(11q23)) where MLL gene located, thus MLL gene was rearranged. Later on, this mutation produced fusion protein. This type of translocation was often noted in acute myeloid leukemia or acute lymphoid leukemia.


KẾT QUẢ XÉT NGHIỆM CÔNG THỨC NHIỄM SẮC THỂ
(KARYOTYPE) TỦY XƯƠNG

Họ và tên: [redacted] Năm sinh: 2019 Giới tính: Nam
Chẩn đoán: Theo dõi BCC/ Viêm phổi Ngày, giờ lấy mẫu: 24/09/2019 08:46:34
Bác sĩ điều trị: [redacted] Ngày, giờ nhận mẫu: 24/09/2019 11:30:34
Nơi gởi bệnh phẩm: BV. NĐTP Chất lượng mẫu: ĐẠT
Bệnh phẩm: Máu Ghi chú:
Người thực hiện: [redacted]

Kỹ thuật: Nhuộm Band G (QT-DTPT-47/48/49/50)
Ngày, giờ trả kết quả: 08/10/2019
Kết quả xét nghiệm:
46,XY,t(9;11)(q34;q23)[6]/46,XY[14]

Diễn giải:

Hình ảnh cụm tế bào

Hình ảnh công thức nhiễm sắc thể

Figure 1. Result of Karyotype test

He was given symptomatic treatments and supportive therapy which included oxygen supply, intravenous antibiotics (cefotaxime and gentamycin at first, then changed into ceftriaxone and vancomycin), packed blood cells transfusion (40ml each time for 3 times in total), and platelet transfusion (50ml each time for 7 times in total).

During our treatment course, he responded poorly and seemed to get worse gradually. He had recurrent febrile episodes when the temperature fluctuated between 38 and 38.5 degrees Celsius. He passed a lot of loose stools about 3-4 times/day, sometimes, he passed stool mixed with fresh red blood or black tarry stool. He had new petechiae on the skin each day while all injection sites were bruised. His abdomen got distended more. Signs and symptoms of respiratory distress were similar to shortness of breath and grunts, persistent productive cough, wheezing at night were prolonged in spite of broad-spectrum and great potent antibiotic therapy. He was bottle-fed about 30ml of milk 6-8 times/day, after eating he vomited about 2-3 times/day.

Based on this diagnosis, with patient's severely compromised overall condition, and infrastructure development of the hospital and family's economic status, we decided to apply supportive therapy. After 37 days in hospital, he was extremely weak with rapid shallow breathing and short periods of apnea. He died when he was 95-day-old.

Child no.2

A 1-month-25-day boy was detected to have acute leukemia after his brother had been diagnosed for about 2 weeks. His mother stated that he had a fever, nonproductive cough and wheezing for about 2 days. She brought him to the hospital, and he was claimed as a case of pneumonia/acute leukemia on the basis of peripheral blood findings.

Physical examination was significant for severe pneumonia and acute leukemia diagnosis. He had recurrent febrile episodes, poor feeding, and remarkable respiratory symptoms, included fast respiratory rate, contraction of chest walls, oxygen saturation of 90% by pulse oximetry and coarse breath sounds. He had pale skin and mucosa, he quickly lost energy and appeared fatigue. He had entire body skin discoloration and mild edema because of subcutaneous hemorrhage. Another symptom was to have petechiae, red blood stool, and bruises around both eyes. Examination showed abdominal distention, collateral circulation, hepatosplenomegaly. Peripheral lymph nodes were palpable, such as inguinal lymph nodes, axillary lymph nodes. He had bulging eyes otherwise known as *exophthalmia, especially the left one.*

Peripheral blood findings showed irregular small red blood cells, no nucleated red blood cell, no target cell; highly elevated white blood cells, blast 90%; low platelet count, platelet morphology and size vary, many giant platelets. Complete blood count indicated that hemoglobin was 4.6 g/dL, hematocrit was 21.4%, platelets were 14.000/mm^3, and white blood cells were 799.200/mm^3 with 3.4% of neutrophils, 29.1% of lymphocytes and 32.2% of basophils.

He was provided symptomatic treatments and supportive therapy which involved oxygen supply, intravenous antibiotics (cefotaxime at first, then added gentamycin, finally changed into ceftriaxone and vancomycin), packed blood cells transfusion (35ml each time for 3 times in total), platelet transfusion (35ml each time for 2 times in total) and frozen fresh plasma transfusion (40ml for once only).

The boy's clinical status progressed downward in a more aggressive pattern than his brother's state. He had recurrent febrile episodes with the temperature over 38.5 degrees Celsius. He couldn't eat well and vomited frequently. His respiratory symptoms included fast respiratory rate, wheezing, contraction of chest walls and coarse breath sounds, got significantly more severe. His signs of bleeding became the most outstanding manifestation with petechiae all over the body, bloody stool, and increasing bruise around both eyes. He also had a lot of swollen lymph nodes. They got bigger quickly, and one or more new nodes can be detected each day. His *exophthalmia condition, especially in the left eye, also continued to evolve and caused struggles in eyeball's movements.*

Concerning the relation to his twin brother in clinical and paraclinical manifestation, we concluded him with acute leukemia. Because he had more aggressive clinical course than his brother and the cost for diagnosing techniques were quite expensive, we decided not to perform any further tests and started a supportive therapy. *After 36 days in hospital,* he got worse rapidly with respiratory failure and severe anemia due to excessive blood loss. He died when he was 89-day-old.

IV. DISCUSSION

Studies of leukemia in monozygotic infant twins have advanced our understanding of both the epidemiology and natural history of leukemia in infants. Since the rate of twin births in the United States is 24.6 per thousand live-born infants, the annual incidence of leukemia in infants is 30 per million [8] and estimated concordance rates in monozygotic twins are from 5% to 25% [3], leukemia in infant twins is rare. In ALL patients *MLL* gene rearrangements are most frequent in infants (80% of cases) and very infrequent in older children (< 2%) [14]. Precursor B-cell ALL is much more common than T-cell disease, accounting for approximately 80% of cases of ALL. AML accounts for 18% of cases of childhood leukemia, and CML is very rare and tends to occur in adolescents. Outside of exposure to ionizing radiation, there are very few environmental factors that have shown a strong link with childhood leukemia. Monozygotic twins have a 10% to 15% concordance rate for ALL.

The child no.1 and the child no.2 who had pneumonia represented an infectious syndrome, included recurrent febrile episodes, lethargy, signs of severe pneumonia (shortness of breath, fast respiratory rate, contractions of the intercostal spaces, and coarse breaths sound in all lung fields). They both had signs and symptoms of anemia (fatigue, poor feeding) and thrombocytopenia (petechiae on skin, purpura at injection sites, and red blood stool). They also had metastatic patterns of leukemia in liver, spleen, lymph node. In addition, child no. 2 had metastatic patterns of leukemia in his eyes (*exophthalmia).*

The child no. 1 was diagnosed depended on the history of the disease, clinical examination and typical paraclinical results. Complete blood count illustrated that white blood cells were 651.900/mm^3 with 40,7% of lymphocyte and 2% of neutrophils; hemoglobin was 7 g/dL, normocytic normochromic red blood cells, and hematocrit was 16,8%; platelets were 9.000/mm^3. Peripheral blood findings showed B-ALL type leukemia with blast cell 80%. The result of FISH confirmed there was a mutation at q23 location on autosome 11 containing MLL gene. Karyotype indicated the chromosome set was 46, XY, t(9;11)(q34,q23)/46,XY. Supportive therapy was applied. He died 2 months after diagnosis and treatment.

However, **the child no.2** didn't have a specific test for diagnosis confirmation such as FISH, karyotype tests, and flow cytometry. He only had peripheral blood smear. *Peripheral blood findings showed* irregular small red blood cells, no nucleated red blood cell, no target cell; highly elevated white blood cells, blast 90%; low platelet count, platelet morphology and size vary, many giant platelets. Complete blood count indicated that hemoglobin was 4.6 g/dL, hematocrit was 21.4%, platelets were 14.000/mm^3, white blood cells were 799.200/mm^3 with 3.4% of neutrophils, 29.1% of lymphocytes and 32.2% of basophils. Because of the concordance in the clinical course and the close genetic connection between monozygotic infant twins, he was also identified as acute leukemia and treated by supportive therapy. He died about 6 weeks after being diagnosed.

V. CONCLUSIONS

MLL in identical twins was a rare case, patients with MLL were otherwise unremarkable. The patient was validated by a review of morphologic and immunophenotyping data. Chromosome and karyotype were confirmed by FISH and Karyotype tests. These twins have provided an extraordinarily rich insight into the natural

history and pathogenesis of pediatric leukemia. They were a vivid illustration for prevalent repetitive dictum in medical research that could be learned from studying rare conditions.

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