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Blood typing and transfusion therapy in a patient with A2 subtype acute myeloid leukemia M2: A case report

Kuang XC *et al.* Blood transfusion for AML patients

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Abstract

BACKGROUND

Acute myeloid leukemia (AML) is one of the most common types of leukemia in adults. However, AML is relatively rare in the population overall, accounting for only about 1 percent of all cancers. Treatment for AML can be very effective for some patients, yet it leaves others with serious and even life-threatening side effects. Chemotherapy is still the primary treatment for most AML, but over time, leukemia cells become resistant to chemotherapy drugs. In addition, stem cell transplantation, targeted therapy, and immunotherapy are currently available. At the same time, with the progression of the disease, the patient may have corresponding complications, such as coagulation dysfunction, anemia, granulocytopenia, and repeated infection, so transfusion supportive therapy will be involved in the overall treatment regime. To date, few articles have reported on blood transfusion treatment options for patients with ABO subtypes AML-M2. Blood transfusion therapy is an important supportive treatment for AML-M2, and accurate determination of patients' blood type is one of the most important steps in the treatment process. In this study, we explored blood typing and supportive treatment strategies for a patient with A2 subtype AML-M2 to provide the basis for treatment for all patients.

CASE SUMMARY

In order to determine the blood type of the patient, serological and molecular biological methods were used for reference tests, and the genetic background was studied to determine the patient's final blood type and select the appropriate blood products for infusion treatment. According to the results obtained by serological and molecular biological methods, the blood type of the patient was A2 subtype; the genotype was A02/001; the irregular antibody screening was negative, and anti-A1 was found in the plasma. According to the overall treatment plan, active anti-infection, elevated cells, component blood transfusion support, and other rescue and supportive treatments were given, and the patient successfully passed the stage of myelosuppression after chemotherapy. Re-examination of bone marrow smears showed that AL was in complete remission of bone marrow signs, and minimal residual leukemia lesions suggested no cells with obvious abnormal immunophenotype (residual leukemia cells $< 10^{-4}$).

CONCLUSION

The infusion of patients with A2 subtype AML-M2 with A irradiated platelets and O washing red blood cells can meet the needs of clinical treatment.

Key Words: ABO blood-group system; A2 subtypes; Blood grouping and crossmatching; Blood transfusion; Acute myeloid leukemia; Atypical blood transfusion

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Core Tip: There has always been a debate on the large-scale transfusion therapy for acute myeloid leukemia (AML). This study provides strong and favorable evidence for

the clinical treatment of blood transfusion. There are few specific reports in the literature on the treatment of blood transfusion for patients with AML-M2 blood type A2, and this study provides a protocol and precedent for the treatment of blood transfusion for patients with rare subtype AML. The study can provide clinical reference data for supporting transfusion therapy in patients with clinically rare blood type leukemia.

INTRODUCTION

Acute myeloid leukemia (AML) is a malignant clone⁵ of myeloid progenitor cells in the hematopoietic system, mainly treated by chemotherapy and hematopoietic stem cell transplantation^[1]. Anemia and thrombocytopenia are the main clinical manifestations of patients with AML, and blood transfusion is the most important treatment strategy for anemia and thrombocytopenia caused by chemotherapy in AML. However, blood transfusions have been associated with immunosuppression and can bring a large number of foreign antigens to the patient. It has been repeatedly³ reported that 55% of patients with type A, B, or AB blood with myeloid malignancies have a proportion of red cells with decreased expression of A or B antigens^[2,3].

It has also been noted that AML restricted blood transfusion treatment could more effectively reduce the risk of infection and improve the prognosis of patients than free blood transfusion treatment^[4]. As a result, when clinicians treat patients with type AML-M2, it is not enough to simply improve the hemoglobin content of patients, but rather, it is vital that patients have longer remission, and that treatment reduces the chance of infection, strengthens organ protection, relieves pain, and ultimately, the patient has improved quality of life. It is well known that subtype A is the most common human ABO blood type, and the main subtypes A1 and A2 account for 99.9% of all blood types. Because the A antigen is only present on the red blood cells of the A2 subtype, there is a small number of anti-antibodies in the serum, apart from the anti-B antibody. In practice, when the traditional serological method is used to identify the A2 subtype, subtype O can easily be mistaken for subtype A2. After the infusion of

abnormal blood products, the infusion is invalid in mild cases. However, severe hemolytic transfusion reaction may occur, leading to shock, disseminated intravascular coagulation, and acute renal failure. Subtype A, A1, and A2 are the most common, while A1 and A antigens have been found on A1 cells, but only A antigens on A2 cells. A2 and A2B individuals account for less than 1% of A and AB individuals because of the fewer A2 sites and lower glycosyltransferase activity compared to A1. Among these sites, 1%–8% of A2 and 22%–35% of A2B have resistance to A1. Therefore, clinically, blood typing of patients with AML is particularly important during extensive long-term transfusion support therapy. The cases of weak ABO antigen frequently appear in male AML patients. Further, the DNA methylation level of the ABO gene promoter in patients with weak ABO antigens are significantly higher than that in patients with normal ABO antigens^[5].

Over recent years, the hematology of patients in need of long-term transfusions has been extensively studied. New genomics approaches provide the ability to routinely select donor units that match the receptor antigen for the first time, rather than ABO/Rh blood group system (RHD), thus helping to reduce non-hemolytic fever, allergic reactions, and occurrence of hemolytic reaction complications^[6]. So far, the main techniques used in laboratory identification of ABO subtypes have been polymerase chain reaction sequence-specific primer (PCR-SSP)^[7], gene sequencing^[8], and similar. However, there are few large-scale laboratories in China for such typing, and the routine serological antibody method is still predominantly used^[6]. Accordingly, the existing methods must be used to accurately identify blood type and to more precisely guide appropriately patient treatment. To date, few articles have reported on blood transfusion treatment options for patients with ABO subtypes AML-M2. In this study, we explored blood typing and supportive treatment strategies for a patient with A2 subtype AML-M2.

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CASE PRESENTATION

Chief complaints

A 44-year-old male patient with a 2-mo course of disease of was treated at the Department of Hematology of the Sichuan Academy of Medical Sciences in the Eastern Branch of Sichuan Provincial People's Hospital (Sichuan Institute of Hematology). He came to our hospital in August 2019 due to systemic fatigue and abdominal distension without an obvious cause.

History of present illness

The patient suffered from weight loss and high skin temperature but was not excessively sweating. He had pale conjunctiva, no yellow sclera, no bilateral pupil constriction, and large and round pupils that were sensitive to light reflection. His superficial lymph nodes were non-palpable and enlarged. No rales were detected in the lungs. The patient had arrhythmias, with 132 beats/minute, and no murmurs. He was ABO positive and the negative stereotypes of the patient were not related to the disease.

History of past illness

He had no history of prior treatment or chemotherapy.

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Personal and family history

The patient had no family history of genetic diseases.

Physical examination

A physical examination showed a temperature of 37°C pulse of 132 beats/min, a respiratory rate of 20 breaths/min, and blood pressure of 91/58 mmHg.

Laboratory examinations

The laboratory blood test results indicated the following: White blood cells: $13.75 \times 10^9/L$ (reference range: $3.50\text{--}9.50 \times 10^9/L$), neutrophils: $4.54 \times 10^9/L$, monocytes: $7.01 \times 10^9/L$ (reference range: $0.10\text{--}0.60 \times 10^9/L$), lymphocytes: $1.38 \times 10^9/L$, red blood cell count: $2.71 \times 10^{12}/L$ (reference range: $4.30\text{--}5.80 \times 10^{12}/L$), hemoglobin: 96 g/L

(reference range: 130–175 g/L), platelet count: 17×10^9 E9/L (reference range: 85–303 E9/L), reticulocyte percentage: 2.84% (reference range: 0.67%–1.92%), mirror inspection: primitive + naive cells: 76%, Auer bodies present. Biochemical results indicated the following: Liver function: Blood sugar: 6.31 mmol/L (reference range: 3.90–6.10 mmol/L), alanine aminotransferase 59 U/L (reference range: 9–50 U/L), total albumin: 68.2 g/L, albumin: 39.7 g/L, lactate dehydrogenase: 1629 U/L (reference range: 120–250 U/L), renal function and electrolytes: Normal. Immune results indicated the following: hepatitis C virus IgG antibody: Positive (consider AML-M2) (reference range: negative); the results of flow cytometry showed that the leukemia was a partially mature type, and the leukocyte differentiation antigens, CD34, CD117, CD13, CD33, and myeloperoxidase were positive, while CD10, CD19, CD7, CD20, and CD14 were negative; karyotype analysis indicated the following: 46, XY, del(9)(q22)^[5]/46, XY^[9], the fusion gene was normal. Comprehensive clinical manifestations and laboratory findings were diagnosed as acute M2 Leukemia.

Imaging examinations

Imaging was not included in this case.

Instruments and reagents

The following experimental instruments were used: ORTHOVISION Max automatic blood group analyzer, Orsondor Medical Equipment Trading (China) Co., Ltd., Shanghai, China; Microlab STAR Line automatic blood group analyzer, Changchun Boxun Biotechnology Co., Ltd., Changchun, China; SLAN-96P Real-time fluorescent quantitative PCR, Shanghai Hongshi Medical Technology Co., Ltd., Shanghai, China; and American Bio-Rad 3130 gene sequencer, Bó Lè Life Medical Products (Shanghai) Co., Ltd., Shanghai, China.

The following experimental reagents were used: ORTHO ABO positive and negative typing and RhD blood typing reagent card (column agglutination method) (lot number: ABR207J), Orsendo Medical Equipment Trading (China) Co., Ltd., Shanghai,

China; Changchun Boxun ABO, RhD blood type testing microcolumn card: (Lot No.: 20200904), ABO blood group anti-typing reagent: Changchun Boxun (Lot No.: 2017040201); Changchun Boxun Irregular Antibody Screening Cells (Lot No.: 20201001); Changchun Boxun Biotechnology Co., Ltd., Changchun, China; ALBA Company RhD (IgM) blood typing reagent (monoclonal antibody) (batch number: v177217), British Alba Biotechnology Co., Ltd.; Beijing Jinhao anti-A, anti-B reagent (batch number: 2016083003), Beijing Jinhao Pharmaceutical Co., Ltd. Company, Beijing, China; and Human anti-A and anti-B serums were made by our laboratory.

ABO blood typing and irregular antibody screening

ABO blood typing: A 2 mL of whole venous blood, with EDTA-K2 for anticoagulation, was taken from patient's wife, parents, and a daughter; $1872 \times g$ were centrifuged for 2 min to do a serological pedigree investigation. ABO blood group was identified by the gel microcolumn method, ¹ an immunological method for the agglutination of erythrocyte antigen and corresponding antibody in the gel microcolumn medium. After adding reagents and specimens, the results were ¹ directly observed by the naked eye or analyzed by blood group meter after centrifugation with special centrifuge or card blood matching system. This method has standardized operation and quantitative sample to ensure the accuracy of the results. Two kinds of ABO blood types were tested by Changchun and Johnson according to the reagents' instructions, and the Jinhao blood typing reagent was used to test positive and negative ABO blood types.

Irregular antibody screening

Irregular antibody, also known as accidental antibody, refers to the anti-A and anti-B antibodies of other blood groups in the serum. Irregular antibodies in other blood group systems can lead to transfusion reactions. Mild cases can cause chills and fever, which can affect the therapeutic effect of the treatment. In severe cases, the incoherent red blood cells will be destroyed, or their life will be shortened, resulting in hemolytic transfusion reactions and endangering the patient's life. Therefore, irregular antibody

detection is necessary. A variety of erythrocyte antigens present on screening cells are used to react with the tested serum. If there is agglutination, there is irregular antibody in the serum. Changchun Boxin and Johnson reagent screening was used for irregular antibody screening.

Anti-H test

A test tube was used to detect the surface H red blood cell membrane.

Test of salivary, blood type and substance and absorption and release

The patient's saliva was collected and tested according to the National Clinical Laboratory Procedures^[10], and the plasma and red blood cells of the patient were also absorbed and released according to the National Clinical Laboratory Procedures^[11].

Options of blood transfusion

As there is still no unified standard for blood transfusion strategies for ABO subtypes in China, the principle of homotypic or compatible infusion is usually followed. According to the relevant provisions of Technical Specifications for Clinical Transfusion and combined with the characteristics of patients with subtype A AML, HB < 60 g/L was injected into suspended red blood cells, PLT < 20 × 10⁹/L was injected into platelets. The process was completed while avoiding the infusion of red blood cell antigens corresponding to the antibodies in the plasma of the recipient and red blood cell antibodies corresponding to the antigens of the red blood cell, thus minimizing the input of the antigens of the abnormal red blood cells to prevent the production of alloantibodies due to the immune reaction. Considering the difficulty in obtaining type A2 blood, the patient was given an infusion of type O washed of red blood cells, and the monitoring during transfusion was strengthened to actively prevent the occurrence of adverse reactions. During the course of the disease, the patient received a total of 32 blood transfusions, including 16 (34 U) infusions of type O washed of red blood cells and 16 (16 U) infusions of type A platelets. The experimental data and clinical

manifestations after blood transfusion suggested that the expected therapeutic effect of blood transfusion was achieved, and adverse blood transfusion reactions such as chills and fever after blood transfusion were not likely to occur. In the later treatment, the blood group serological test results were compared with those before transfusion, and anti-A1 was not detected. After the blood transfusion, the patient's hemoglobin was maintained at about 90 g/L, and platelet increased to more than $50 \times 10^9/L$.

The patient's blood transfusion process was smooth, with no rash, fever, lumbago, soy-colored urination, chills or other symptoms. After the infusion, the patient's fatigue, soft limbs, tired and tightness of the heart were significantly relieved, the complexion and mucosa were rosy, the hemoglobin was significantly increased, monitoring showed no significant increase in the reexamination of the reticulata, bilirubin was normal, urochologen was negative, and the lactate dehydrogenase did not decrease.

ABO blood group identification is extremely important in clinical transfusion practice. Blood group identification errors lead to incorrect transfusion of blood products and often serious transfusion reactions. Therefore, in the case of inconsistency between positive and negative serotypes, it is suggested to further improve the detection method and recommend the supplementation of molecular biological methods to avoid blood group identification errors as often as possible, to ensure the safety of transfusion.

ABO gene amplification and sequencing

For exons 6, 7 of ABO genes, partial 5 and 6 introns were amplified and sequenced^[12]. First, DNA was extracted from the samples (DNA concentration between 30–50 ng/mL with A260/A280 values ranging from 1.8 to 2.0). The PCR instrument was amplified, and the amplified products were purified and sequenced according to the instructions of the kit.

Cross-matching test

Since a low titer anti-A1 was found in the patient, O washing red blood cells were used for cross-matching test.

Results

The results of the ABO blood typing serological test showed that the ABO of the patient was not consistent, the erythrocyte and anti-A, anti-AB were agglutinated, the plasma and A1 erythrocyte were weakly agglutinated, and the B cells were agglutinated. The ABO blood type and anti-H results of the patient's parents, wife, and daughter were normal (Table 1). Two reagent irregular antibody screening for antigen spectra response in three cells was negative (Table 2)

Test of salivary, blood type and substance and absorption and release of the patient

The results showed secretory substances A in the patient's saliva substance (Table 3).

ABO gene test results of the patient

The results of the ABO testing indicated that the individual was A 02/O01 genotype. In sequencing ABO exons 6 and 7, we found that the changes were single base substitution (C/T) at 467 and C deletion at 1061 (467C>T, 1061del C).

FINAL DIAGNOSIS

Acute myeloid leukemia M2.

TREATMENT

In addition to conventional chemotherapy, O-type washed red blood cells and isotype irradiated platelets were used as adjuvant therapy.

After admission, the pathological *in vitro* antitumor drug sensitivity assay bioluminescence assay was conducted; after which, the daunorubicin combined with cytarabine to induce remission for AML (DA) regimen chemotherapy (daunorubicin 100mg qd d1-d3, cytarabine 200mg qd d1-d7) was started,

simultaneously with the symptomatic treatments such as esomeprazole gastric, liver, and heart protection, hydration, and alkalization.

On September 9, 2019, the bone marrow morphology was reexamined, revealing complete remission. On September 12, 2019, lumbar puncture and intrathecal injection were performed, and cerebrospinal fluid flow indicated no abnormality. The patient had long myelosuppression, heavy infection, and difficulty in blood transfusion after the previous chemotherapy, so the reduction of dosage was given in this consolidation chemotherapy regimen. CD14 was not expressed, and the patient was treated with cytarabine 1g q12h d1, d3, and d5 on September 13, 2019. After two chemotherapy treatments, the patient developed grade IV myelosuppression, granulocytopenia, recurrent high fever, severe lung infection, and cardiac insufficiency. After active treatment, the patient's condition gradually improved and was discharged with medication.

OUTCOME AND FOLLOW-UP

Supportive care brought the patient's condition into remission. The patient's condition was stable throughout the maintenance treatment period, which provided a basic reference for follow-up stem cell transplantation.

DISCUSSION

There are currently few international reports on ABO subtype patients with blood diseases. It is generally accepted that the phenomenon of ABO antigen weakening occurs occasionally in patients with leukemia, which is likely to lead to inconsistent positive and negative stereotypes in blood group identification. It is undoubtedly the best choice for blood donors of ABO subtype patients, but it is difficult to do in practice. When considering transfusion treatment for ABO subtype leukemia patients, we should avoid importing red blood cells containing red blood cell antigens corresponding to antibodies in the plasma and plasma containing antibodies corresponding to red blood cell group antigens and choose type O washing red blood cells and normal plasma

corresponding to subtype, cryoprecipitate and platelets for cross matching^[13,14]. This case report illustrated that the blood typing of patients with subtype A AML should follow the principle that positive and negative typing results should be consistent, and the specimens should be tested for molecular biology if they are not. Patients for whom it is difficult to obtain type ABO blood type and need blood transfusion treatment should be strictly treated according to the blood transfusion regulations to ensure efficacy and avoid adverse reactions. However, few articles report on blood transfusion treatment options for patients with ABO subtypes AML-M2. As for treatment strategies for patients, it is necessary to ensure that before deciding on the treatment, the blood type of the patient is determined to exclude any correlation with the disease or chemotherapy that could reduce the content of antibodies in the blood. If a patient has anti-A1 with A2 AML-M2, he or she should not be treated with regular homotypic infusion, as the infusion of the same type of red blood cell suspension or washing of red blood cells in the presence of anti-A1 antibodies in the patient's body can only aggravate the patient's condition. Consequently, it is very challenging to achieve the routine infusion for this subtype. If the symptoms are mild, a patient might also experience fever and chills, and in severe cases, this may lead to hemolytic transfusion reactions, or could lead to death.

Presently, there are 39 blood type systems reported by International Blood Transfusion Association^[15]; among these, ABO are of great significance in the field of genetic research, clinical blood transfusion, and transplant immunity. The most important subtypes of the ABO blood type system are the A1 and A2 subtypes, which do not belong to any specific blood type. The identification of subtypes of ABO blood type in clinical laboratories in China is still mainly based on the traditional serological methods, considering such an approach is economical, fast, and easy to standardize. However, the agglutination intensity of antigens and antibodies tends to change in different subtypes, easily leading to missed tests or false identification. The perfect matching of patient and donor blood type is achieved by genotyping, which makes up for the standard that traditional serology cannot reach, but it is difficult to popularize

on a large scale due to high cost and technical issues. Although this method increases the patient's expenditure to a certain extent, it has many advantages for the patients' long-term prognosis.

The use of serological methods to identify ABO subtypes should be based on the agglutination intensity between red blood cells and ¹anti-A, anti-A1, anti-B, anti-AB, and anti-H, the presence of anti-A1 in plasma and the A, B, and H substances in the saliva of secreted human^[16]. In our practical clinical work, subtype blood types are generally found in ABO blood group identification. When the positive and negative stereotyping results are inconsistent, or the agglutination intensity is weak, methods other than routine tests should be carried out for identification, such as anti-H, anti-A1, anti-A, and anti-B serums that are increased in positive stereotyping; increase O cells, A1 cells, A2 cells, absorption and release tests, and saliva blood group substances detection^[17]. Accurate identification of blood type is an important prerequisite for blood transfusion therapy for patients with AML-M2. Based on the correct blood group and the patient's own body antibodies, the homotypic transfusion of suspended red blood cells or washing of red blood cells can be excluded, and the best scheme selected. In the present study, the transfusion plan for the patient with A2 subtype (M2) included infusion of O-type scrubber red blood cells and irradiated platelets of the same blood type (A-type), which achieved satisfactory symptom relief effect.

The main treatment for patients with A2 subtypes AML-M2 is still chemotherapy, whose main disadvantages are long duration and serious side effects. Blood transfusion is the main treatment for addressing anemia caused by chemotherapy and an important measure for prolonging the patient's life. Hence, ABO blood typing is of crucial importance. In this study, we selected O washing of red blood cells as the transfusion red blood cells of patients to avoid the existence of antibodies corresponding to red blood cell antigens and antibodies corresponding to red blood cell blood group antigens in plasma. We selected homo-irradiated platelets for transfusion to prevent the occurrence of incompatibilities between antibodies in plasma or ABO antigen on platelets and the recipient.

The pre-transfusion test of patients with A2 subtype AML-M2 should follow the principle that the positive and negative results of blood typing must be the same, and the different samples should be tested by molecular biology. For patients with difficult ABO blood type setting and in need of blood transfusion treatment, the treatment should be carried out strictly according to the relevant regulations of blood transfusion to ensure the curative effect and avoid adverse reactions. Consequently, when making a clinical blood transfusion plan, it is necessary to consider all kinds of blood type systems, diseases, physical quality, blood safety problems, and similar. In any possible case, the treatment should be local to improve the future treatment plan^[18].

CONCLUSION

The infusion of patients with A2 subtype AML-M2 with A irradiated platelets and O washing red blood cells can meet the needs of clinical treatment. With ABO blood typing, it is necessary to uphold the principle of consistency between positive and negative results, while molecular biological methods should also be used to test specimens that do not conform to this principle. For patients who may be affected by the weakening of antigens due to ABO blood diseases and who require blood transfusion before the ABO blood group is determined, the strictest standards for blood transfusion therapy should be adopted to ensure the efficacy of blood transfusion and to avoid the occurrence of accidental antibodies that may affect subsequent blood transfusion therapies. In clinical practice, homotype infusion is ideal, but in clinical practice, it is difficult to meet the homotype transfusion blood products for patients with this subtype. In the case of limited conditions, and to avoid hemolysis reaction, isoantibody production, and heterosexual blood transfusion whenever possible, it is best to transfuse O-type red blood cells or blood products corresponding to the same subtype for cross-matching blood. All of the above treatment presupposes that we can accurately determine the current blood type of the patient, so assessment of the accurate blood type becomes particularly important. Using molecular biological sequencing or PCR-SSP to identify the patients' blood type will gradually become the consensus of

clinicians and transfusion doctors. This consensus could lead to safer treatment strategies for ABO subtype leukemia patients.

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