CASE REPORT

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Reduced ABO blood group antibody titers in patients after CD19 CAR-T cell therapy

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1. INTRODUCTION

With rapid developments in genetic engineering, tumor immunology, and cellular engineering, chimeric antigen receptor T cell (CAR-T) cell therapy has become a novel immunotherapy for oncology and other medical fields.[1] The promising results of CD19 CAR-T treating B-cell malignancies were reported.[2,3] Simultaneously, there existed many adverse events, the most reported of which including B-cell aplasia, hematological toxicity, cytokine release syndrome (CRS), and immune effector-cell–associated neurotoxicity syndrome (ICANS),[3,4] but there is still lack of reports demonstrating the impact of CD19 CAR-T on the ABO blood group potency of patient's serum. Blood transfusion plays an important role in treating diseases, especially in treating hematological diseases, and the accurate identification of ABO blood groups is a prerequisite for the safe blood transfusion. Meanwhile, the valid measurement of patient's serum ABO blood group antibody potency is essential for the identification of the patient's ABO blood group type. In this case report, we summarized the data of 10 patients receiving CD19 CAR-T cell immunotherapy in our hospital in recent years and had their potency measured after treatment, with a view to conducting a preliminary analysis of the impact of CD19 CAR-T cell therapy on the ABO blood group antibody potency in patients' serum.

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L.Q. and W.Y. designed the research and wrote the manuscript. Y.Z., W.S., L.W., H.W., Z.G., L.F., C.X., G.X., L.Y., and W.J. interpreted the results. F.K., S.J., S.Y., Z.X. acquired clinical data, collected the samples, performed laboratory investigations, and interpreted data. All the authors critically revised the manuscript and approved the final version.

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2. CASE REPORTS

We enrolled 10 patients with hematological malignancy and receiving CD19 CAR-T in our hospital from November 2017 to January 2021 in this study, including 9 cases with acute lymphoblastic leukemia and 1 case of acute leukemia of ambiguous lineage. Six males and 4 females out of 10 patients, and median age was 36.5 years old (8–45 years). Four patients with blood type A, 3 patients with blood type B, and 3 patients with blood type O. All patients received lymphodepletion chemotherapy with FC protocol within 1 week before CD19 CAR-T infusion (intravenous fludarabine at 25–30 mg/m^2 daily from day 4 to 2; and intravenous cyclophosphamide at 350 mg/m^2 daily on day 4 and 2; and intravenous cytarabine at 100 mg/m^2 per day from day 4 to 1). Eventually, 9 patients had their peripheral blood ABO antibody potency measured within 1 to 3 months after receiving CAR-T cells, and 1 patient had his peripheral blood ABO antibody potency measured within 6 months after receiving CAR-T cells (Table 1).

The anti-B potencies in the 4 patients' serum with blood group "A" were 2, 4, 8, 8. The anti-A potencies in the 3 patients' serum with blood group "B" were 4, 4, 16. The anti-A and anti-B potencies in the 3 patients' serum with blood group "O were (A: 16, B: 4), (A: 32, B: 8) (A: 16, B: 16) (Table 1). However, both anti-A and anti-B potency were above 128 in the serum of normal humans.[5] Consequently, lymphodepletion with FC regimen and infusion CD19 CAR-T may lead to a decrease of the anti-A and anti-B potencies in the serum of patients.

The majority of ABO blood type antibody in serum was immunoglobulin M, and B lymphocyte plays a significant role in producing immunoglobulin. Hence, we hypothesized that the decline of ABO antibody potency in serum may relate to the decreasing of IgM and B-cell percentage. In our study, all 9 patients showed a decreasing trend, except for patient no. 6 who showed a decrease and then an increase in IgM (**Fig. 1A**). Meanwhile, all 9 patients showed a decreasing B lymphocyte percentage, except for patient no. 6 who showed a decreasing and then increasing B lymphocyte percentage (**Fig. 1B**).

In previous studies, a correlation was found between the impact of CD19 CAR-T cell therapy and CD19-negative immune escape[6]. However, it is not clear if there is a correlation between antibody potency and the impact of CD19 CAR-T cell therapy. Furthermore, we analyze the association between clinical outcome and the ABO blood group antibodies potency. When measuring antibody potency, 6 patients achieved complete remission (CR), with 2 patients in CR with incomplete hematological recovery and 2 patients relapsing after remission (Table 1). However, in patients receiving CD19 CAR-T cells immunotherapy, there was no significant causal relationship between serum ABO blood group antibody potency and their clinical outcome Table 1 and **Fig. 2**.

Table 1

Results of ABO blood group potency in peripheral blood of 10 patients.

Patients no.	Gender	Age	Disease	ABO blood type	ABO antibody potency	Time of measuring	Patient status at time of titer determination
1	Female	44 y	ALL	A	-A: 0; -B: 2	1 mo and 24 d	CR
2	Male	8 y	ALL	B	-A: 4; -B: 0	1 mo and 16 d	CR
3	Male	42 y	ALL	A	-A: 0; -B: 4	1 mo and 3 d	CRi
4	Female	38 y	ALL	B	-A: 16; -B: 0	2 mo and 2 d	CRi
5	Male	15 y	ALAL	O	-A: 16; -B: 4	1 mo and 5 d	CR
6	Male	19 y	ALL	O	-A: 32; -B: 8	2 mo and 24 d	CR
7	Female	45 y	ALL	A	-A:0; -B:8	2 mo and 20 d	relapse after remission
8	Female	40 y	ALL	A	-A: 0; -B: 8	2 mo and 29 d	CR
9	Male	34 y	ALL	B	-A: 4; -B: 0	2 mo and 26 d	CR
10	Male	35 y	ALL	O	-A: 16; -B: 16	5 mo and 22 d	Relapse after remission, followed by CD22 CAR-T cell infusion 4 mo later, relapse again after remission

ALAL = acute leukemia of ambiguous lineage, ALL = acute lymphoblastic leukemia, CRi = complete remission with incomplete hematological recovery, CAR = chimeric antigen receptor, CR = complete remission.

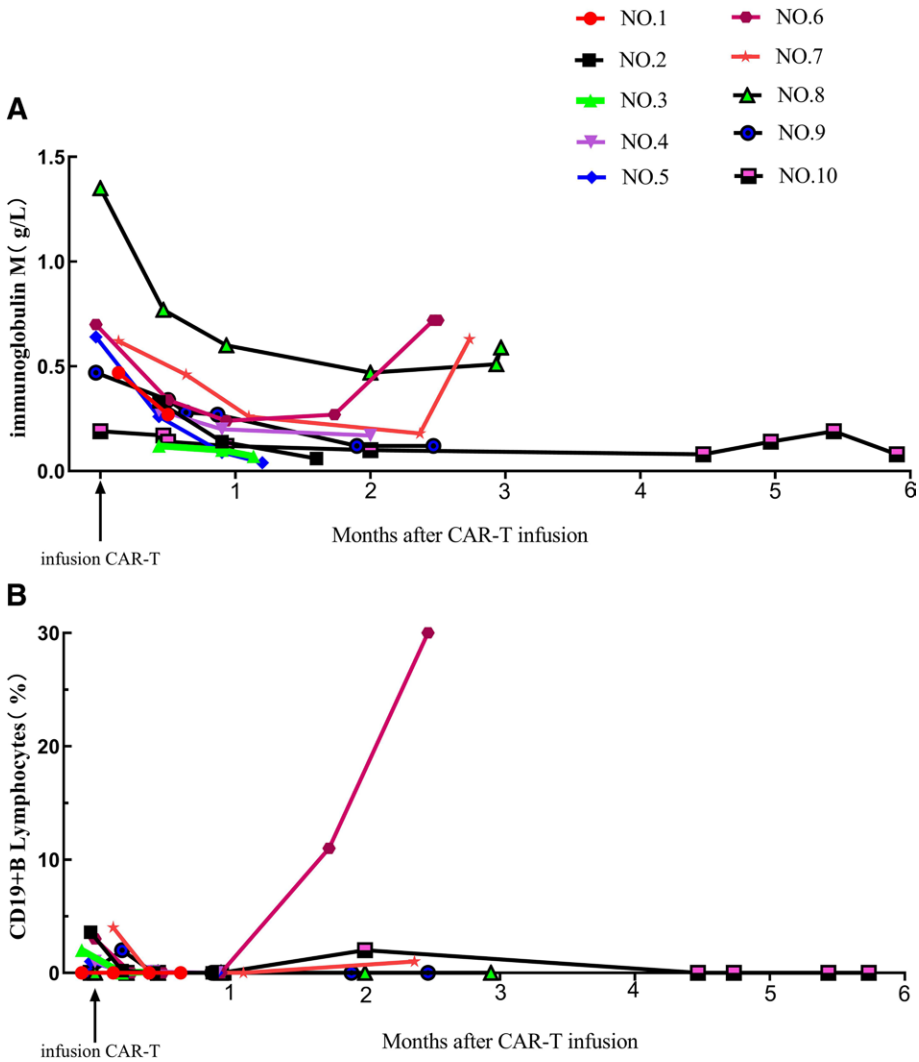

Figure 1. Trends in IgM (A) and B lymphocytes (B) over the period from the infusion of anti-CD19 CAR-T cell therapy to the time of post-infusion ABO blood group antibody potency measurement. CAR-T = chimeric antigen receptor T cell.

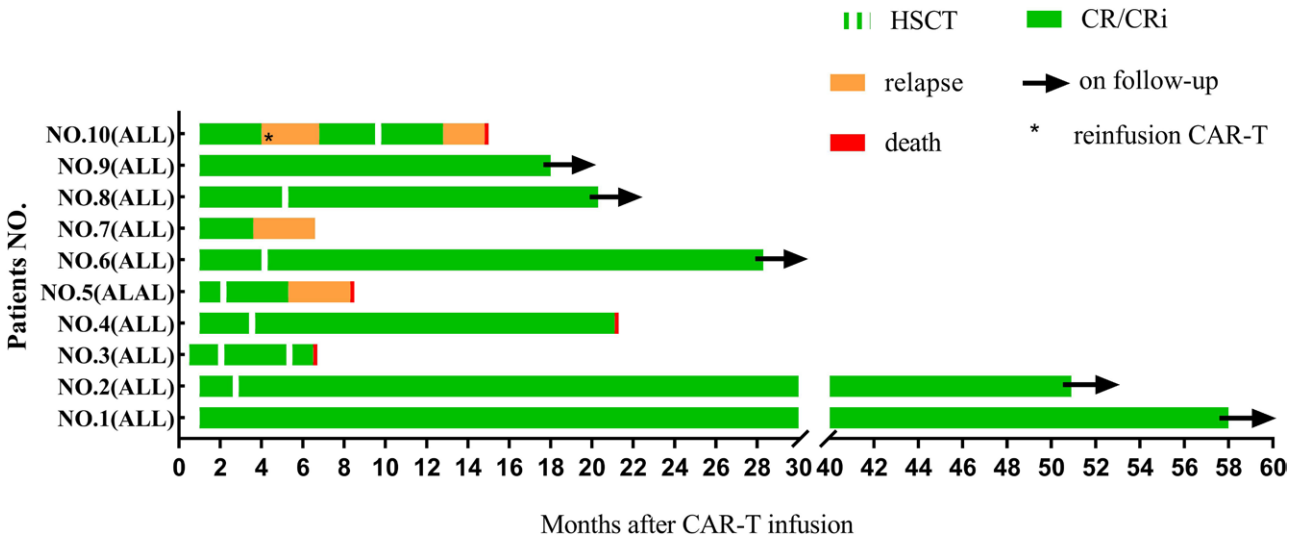

Figure 2. Therapies (reinfusion CAR-T for "*" and HSCT for green and white intermixed colors) and clinical outcomes (complete remission and complete remission with incomplete hematological recovery for green; relapse for orange and death for red) are represented concerning time in months. CAR-T = chimeric antigen receptor T cell. HSCT = hematopoietic stem cell transplantation.

3. DISCUSSION

Blood transfusion is crucial in treating diseases, especially in treating hematological diseases, and the accurate identification of ABO blood groups is a primitive requirement for the safe blood transfusion, which is strongly associated with serum antibody potencies. In this case report, we found a decrease of ABO antibody potency in patients with malignant hematological disease who received CD19 CAR-T. The highest potency of antibody is 32 after receiving CD19 CAR-T in our patients. It has been demonstrated that ABO antibodies potency in children basically reaches adult levels by the age of 5 to 10 years, and even though it decreases with age, the median potency of antibodies in the serum of people in their 90s reaches 128.[5]

Interestingly, we also found a reduction trend in B lymphocyte and IgM. As we all know, one of the adverse events of CD19 CAR-T cell therapy is B-cell dysplasia,[7,8] leading to hypoglobulinemia. After 1 week of CD19 CAR-T infusion, CD19-positive B cells were exhausted, and B-cell dysplasia may persisted for more than 1 month.[9] Hence, B-cell dysplasia is likely to contribute the reduction of ABO blood group antibody potency. Second, the effect of accumulated immunosuppression following previous treatment like lymphodepletion before CAR-T infusion,[10] and some specific previous underlying condition, such as patients suffering from B-ALL,[10,11] may also contributed to the lymphopenia. After stimulated by blood type antigen, B cell is activated and differentiates into plasma cells to produce immunoglobulins (Ig) that bind specifically to the corresponding antigen and elicit an immune response, which is known as erythrocyte blood group antibodies. ABO blood group antibody consist of IgG, IgM or IgA, but IgM predominates. Hence, it is tentatively concluded that the reduced potency of ABO blood group antibodies in patients with malignant hematological diseases receiving CD19 CAR-T cells may be a result of various reasons, most likely due to a reduction in the number of B lymphocytes and IgM after CAR-T cell therapy, which may leading to a reduction in the serum ABO blood group antibodies potency.

Furthermore, reduced blood group antibody potency can lead to difficulties in blood group identification, affecting blood transfusion safety. Firstly, the current international method of blood group identification is to combine the forward type and reverse type. In ABO blood grouping determination, the level of agglutination needs to reach 2+. When the antibody potency is low, the level of agglutination of reverse type may not reach 2+ or may even be negative, which may result in a discrepancy between forward type and reverse type and may make it difficult to identify the patient's blood group or may make an incorrect identification. Secondly, although most patients had confirmed ABO blood group before CAR-T treatment, when these patients return to other hospitals for pre-transfusion testing or when they come for further treatment after CAR-T treatment, there is a high risk that their blood type may not match the forward or reverse type, which may result in these patients not being able to receive a transfusion promptly. It is, therefore, essential to know these patients' medical and treatment histories. Even though, increasing the serum volume, absorption and dispersion tests and genetic sequencing can be effective to identification blood group accurately. The validity of these techniques for patients receiving CD19 CAR-T are not yet clear.

In conclusion, CD19 CAR-T therapy can reduce the ABO blood group antibody potency by decreasing B lymphocytes and the amount of IgM immunoglobulin production. In contrast, in patients receiving CD19 CAR-T cells, there was no significant causal relationship between serum ABO blood group antibody potency and their clinical outcome. Further trials are warranted to better understand the mechanism of the reduction of ABO blood type antibodies to improve blood transfusion safety for patients receiving CD19 CAR-T.

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