Research Paper

The Interference of Monoclonal Anti-Cluster of Differentiation 47 on Transfusion Compatibility Testing

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Hu5F9-G4 and AK117, novel cluster of differentiation 47 blockers, are used to treat solid tumors and hematoma. Because cluster of differentiation 47 is expressed on erythrocytes, treatment with monoclonal anti-cluster of differentiation 47 may interfere with the results of transfusion compatibility testing. This study analyzed the interference and treatment of Hu5F9-G4 and AK117 on transfusion compatibility testing. Saline and microcolumn gel methods were used to identify the blood types of the patients. To screen irregular antibodies, salt water, indirect antiglobulin test and polyamine methods were used. Various methods were used to identify antibodies, including saline, indirect antiglobulin test, polyamine and acid diffusion. This paper explores current methods for removing interference caused by monoclonal anti-cluster of differentiation 47. Hu5F9-G4 interferes with reverse typing of ABO blood groups, resulting in agglutination of all reagents used in antibody screening and identification. Patients' erythrocyte acid dispersive fluid reacted positively with spectrum cells and erythrocytes treated with papain reacted positively with serum. In saline and coagulant amine media, AK117 did not agglutinate with screening cells, but it did with anti-human globulin media. Pre-transfusion testing can be interfered with by anti-cluster of differentiation 47 antibodies. To ensure safe blood transfusions, patients should have their blood type tested and antibodies screened before treatment.

Key words: Cluster of differentiation 47, Hu5F9-G4, AK117, transfusion, compatibility testing, immunoglobulin

Cluster of Differentiation 47 (CD47), known as integrin-associated protein, is a cell surface transmembrane glycoprotein that belongs to the immunoglobulin superfamily and is expressed in all cells (including erythrocytes and platelets) and is highly expressed on a variety of tumor cells[1-3]. CD47 inhibits the phagocytic activity of macrophages by binding to Signal-Regulated Protein Alpha (SIRPα) ligands on the surface of macrophages, sparing tumor cells from clearance and thus evading immune system attack[4-6]. In recent years, CD47 as a novel therapeutic target, has become a research hotspot for the treatment of hematological diseases and solid tumors by blocking the CD47-SIRPα signaling pathway through anti-CD47 antibodies and promoting phagocytosis of macrophages to remove tumor cells[7,8]. A variety of anti-CD47 antibodies have entered the clinical trial stage at China and abroad[9-11], such as: Gilead's Hu5F9-G4, a monoclonal Immunoglobulin G4 (IgG4) antibody, has certain blood toxicity; Kang Fang independently developed a new generation of CD47 monoclonal antibody AK117 (IgG4 antibody), the current clinical trials have confirmed that AK117 has more outstanding safety advantages and strong phagocytic activity than other CD47 monoclonal antibody. Since CD47 is also expressed on red blood cells and platelets, treatment with CD47 monoclonal antibody is not only likely to cause anemia and thrombocytopenia, but also interfere with the results of transfusion compatibility testing[9]. Currently, there are few cases of CD47 monoclonal antibodies interfering with pre-transfusion testing and there is a lack of experience with difficult blood matches due to CD47 monoclonal antibodies. Here, we performed transfusion compatibility testing in patients treated with different types of CD47 monoclonal antibodies, analyzed the characteristics of drug antibody interference testing and explored available solutions with reference to the literature. A 37 y old male (patient 1), was diagnosed with multiple myeloma, had a history of multiple blood transfusions and was taking CD47 monoclonal antibody (Hu5F9-G4) regularly. A 60 y old female (patient 2), was diagnosed with acute myeloid leukemia, had a

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history of multiple blood transfusions and was regularly treated with CD47 monoclonal antibody (AK117). Centrifuge for microcolumn gel card (TD-A, Changchun, China) and centrifuge used for blood bank (Baso). A blood grouping reagent against A and B (production lot 20210608) was purchased from Shanghai Blood Biomedical Co. Anti-D blood grouping reagent (production lot number: 517219) purchased from Yimecon Biological Products Co. From Shanghai R&P Biotechnology Co., a Rhesus (Rh) blood group antigen test card was purchased (production lot number: 10211106). Human ABO blood group anti-stereotypic red blood cell reagent (production lot number: 20225321), antibody screening red blood cell reagent (production lot number: 20227021), irregular antibody identification red blood cell reagent (production lot number: 20220629) purchased from Shanghai Blood Biomedical Co. Multispecific anti-globulin reagent, monospecific anti-IgG, monospecific anti-C3d (production lot number: 20215001, 20205101, 20215201, respectively) purchased from Shanghai Blood Biomedical Co. Acid dispersion reagent (production lot number: 20212101) purchased from Shanghai Blood Biomedical Co. ABO blood group test was performed by saline method; Rh blood group test was performed by microcolumn gel method. In order to check irregular antibodies on the surface of red blood cells, the Direct Antiglobulin Test (DAT) test was conducted in a test tube. Several antibody screening methods were used to determine the type of antibody, including the saline method, Indirect Antiglobulin Test (IAT) method and condensed amine method. In order to identify antibody specificity, the saline method (2 times centrifugation, 37° reaction), IAT method, coagulant amine method and acid dispersion test were used. The relevant operations were carried out according to the reagent instructions. In a tube, add 1 ml of the patient's red blood cells and wash them 4 times, add an equal amount of reagent A, mix well and centrifuge for 1 min, take the supernatant and add reagent B, adjust pH to 7 and proceed with the test. Plasma from the patient was diluted by multiple ratios and the antibody titer was determined using a microcolumn gel card. Before CD47 monoclonal antibody treatment, ABO blood typing of two patients was consistent with positive and negative typing. Patient 1 was type "A" with Rh typing CcDEe and patient 2 was type "O" with Rh typing CcDEe, both of whom had negative DAT tests as shown in Table 1 and Table 2. After treatment with CD47 monoclonal antibody, the ABO blood group test of patient 1 was inconsistent, the positive type was still "A", while the negative type was full agglutination. The blood type of patient 2 is always "O". Rh blood typing of 2 patients was not disturbed and DAT test was positive as shown in Table 1 and Table 2. Before receiving CD47 monoclonal antibody treatment, the irregular antibody screening results of both patients were negative. Different methods were used to detect irregular antibodies of patients after using CD47 monoclonal antibody. The results are shown in Table 3. Patient 1 irregular antibody in saline, IAT and coagulant amine assays showed total agglutination. Patient 2 was negative in saline method and coagulant amine method, but was fully agglutinated in IAT method. The serum and spectrum cell responses of patients after treatment with CD47 monoclonal antibody are shown in Table 4. The spectrum cells fully agglutinate with the serum of patient 1 in saline medium (including 37°), antiglobulin medium and polyamine medium. The red blood cells of patient 1 were treated with acid-releasing reagent and the spectral cell reaction was still completely aggregated in antiglobulin medium. The serum and spectrum cells of patient 2 were negative in saline medium and condensed amine medium, but all positive in IAT method. After the red blood cells were treated with acid-releasing reagent, they were weakly positive in saline medium, and the intensity increased slightly after secondary centrifugation in saline medium. The intensity of antiglobulin method was 2+s. Studies have reported that CD47 monoclonal antibody interferes with transfusion compatibility testing such as blood group identification, antibody screening and cross-matching, making pre-transfusion testing difficult[12]. In this study, pre-transfusion tests were normal in 2 patients before CD47 monoclonal antibody treatment, but after treatment, transfusion compatibility tests were disturbed in both patients and treatment with different types of CD47 monoclonal antibody had a differential effect on transfusion compatibility tests. Because CD47 monoclonal antibody binds to the patient's red blood cells, it leads to a positive direct anti-human globulin test, which may cause false agglutination in ABO blood group identification positive typing. In addition, the ABO blood group reverse typing test of non-"O" patients treated with CD47 monoclonal antibody may be completely agglutinated, so it is difficult to judge ABO blood group[9,11]. In this study, patient 1 is type "A", and ABO reverse agglutination after the application of Hu5F9-G4 monoclonal antibody may be related to the higher antibody titer in the patient's serum. Patient 2 was type "O", and DAT test was positive after application of AK117 monoclonal

antibody, but no pseudo coagulation was observed in the ABO orthotypes. In irregular antibody screening and identification assays, Hu5F9-G4 monoclonal antibody interfered with saline, IAT and coagulant amine assays with strong agglutination. It was found that when the potency of anti-CD47 antibodies in patients was as high as 4096-16 384, agglutination occurred when antibody screening was performed in saline media[13]. Antibody potency testing in patient 1 treated with Hu5F9-G4 monoclonal antibody was performed with potencies up to 16 384, which is consistent with the finding in the literature. In this study, AK117 monoclonal antibody did not show agglutination in the screening and identification of antibodies in saline medium and polyamine medium, but agglutinated in antiglobulin medium. It is speculated that the titer of AK117 monoclonal antibody in patient 2 is lower or the influence of AK117 monoclonal antibody on blood transfusion compatibility test is less than that of Hu5F9-G4 monoclonal antibody. Pineapple enzyme, papain, fig protease, trypsin and dithiothreitol do not destroy CD47 antigen and cannot mitigate interference with transfusion compatibility testing[14]. In this study, antibody screening remained positive after treatment of erythrocytes with papain in patient 1, which is consistent with the literature. Various methods have been proposed to remove CD47 monoclonal interference, such as allosteric adsorption, Gamma-clone® and solid phase agglutination[11,13]. The allosteric adsorption is a time-consuming and ineffective method that uses red blood cells or platelets to remove anti-CD47 antibodies by multiple uptakes, the number of uptakes being related to the potency of the drug antibodies in the serum. There are limitations in practical work because homologous antibodies may be missed by red blood cell uptake and large numbers of platelets may not be available by platelet uptake. Because CD47 monoclonal antibodies are of the IgG4 type, they can be removed more quickly and effectively using the Gamma-clone® antiglobulin reagent (lacking detection of IgG4 antibodies) or the Immucor Capture-R solid phase agglutination method, but the cost is high and difficult to spread. At the beginning of anti-CD47 antibody therapy, patients often develop anemia and thrombocytopenia and need to receive blood transfusions[15,16]. Therefore, it is recommended to perform ABO/Rh blood group identification (genotype testing if available), irregular antibody screening test and platelet antibody screening on patients before using CD47 monoclonal antibodies and to perform matched transfusion based on these results to ensure the safety of transfusion for patients[17].

TABLE 1: RESULTS OF BLOOD TYPING

		-A	-B	Ac	Bc	Oc	Self	-D	-E	-C	-e	-c	ABO	Rh
Patient 1	Before	4+	0	0	2+	0	0	4+	4+	4+	4+	4+	A	CcDEe
	After	4+	0	1+	2+s	1+w	0	4+	4+	4+	4+	4+		
Patient 2	Before	0	0	3+	3+	0	0	4+	4+	4+	4+	4+	O	CcDEe
	After	0	0	3+	3+	0	0	4+	4+	4+	4+	4+		

Note: (s): Enhancement and (w): Weakening

TABLE 2: RESULTS OF DAT TEST

Sort		DAT test			
		Broad-spectrum IgG	Monoclonal antibody IgG	Monoclonal antibody C3	Saline control
Patient 1	Before	0	0	0	0
	After	1+w	1+w	0	0
Patient 2	Before	0	0	0	0
	After	3+	2+	0	0

Note: (w): Weakening

TABLE 3: ANTIBODY SCREENING RESULTS AFTER CD47 MONOCLONAL ANTIBODY TREATMENT

Method		Screening cells			Self
		S1	S2	S3	
Patient 1	Saline	2+s	2+s	2+	0
	IAT	2+s	2+s	2+	1+w
	Poly	3+	3+	2+s	2+
Patient 1	Saline	0	0	0	0
	IAT	2+	2+	2+	2+s
	Poly	0	0	0	0

Note: Enhancement and (w): Weakening

TABLE 4: PATTERN OF SPECTRUM CELL RESPONSE AFTER CD47 MONOCLONAL ANTIBODY TREATMENT

Method		Spectrum cell										Self
		1	2	3	4	5	6	7	8	9	10	
Patient 1	Saline	1+s	2+	2+	2+	2+	1+s	2+	2+	2+	2+	0
	37°	3+	3+w	3+	3+	3+	3+w	3+	3+	3+	3+	0
	IAT	2+	2+	2+s	2+s	2+s	2+	2+s	2+s	2+s	2+s	1+w
	Poly	2+s	2+s	3+	3+	3+	2+s	3+	3+	3+	3+	2+
	Elution test (IAT)	3+s	3+s	3+s	3+s	3+s	3+s	3+s	3+s	3+s	3+s	3+s
Patient 2	Saline	0	0	0	0	0	0	0	0	0	0	0
	IAT	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+
	Poly	0	0	0	0	0	0	0	0	0	0	0
	Elution test (saline)	±	±	±	±	±	±	±	±	±	±	0
	Elution test (IAT)	2+s	2+s	2+s	2+s	2+s	2+	2+s	2+s	2+s	2+s	2+s

Note: (s): Enhancement and (w): Weakening

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Conflict of interests:

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