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Research Article

Study on the Missed Detection of Unexpected anti-E by MGT and Polybere Method

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Abstract:

The mitogen-activated protein kinase kinase (MEK) pathway is a key regulator of cell proliferation, survival, and differentiation, and its dysregulation is closely linked to hepatocellular carcinoma (HCC) development. This retrospective study aimed to investigate the expression of MEK in HCC, its association with clinicopathological features, and prognostic significance. A total of 260 HCC patients who underwent surgical resection at our institution from 2016 - 2021 were included. MEK expression in tumor and adjacent non-tumor tissues was detected by immunohistochemistry. High MEK expression in tumor tissues was significantly associated with larger tumor size ($p = 0.003$), higher histological grade ($p = 0.01$), microvascular invasion ($p = 0.001$), and elevated alpha-fetoprotein (AFP) levels ($p = 0.005$). Multivariate Cox regression analysis identified high MEK expression as an independent predictor of poor overall survival (hazard ratio [HR] = 2.2, 95% confidence interval [CI]: 1.4 - 3.5, $p < 0.001$) and recurrence-free survival (HR = 2.1, 95% CI: 1.3 - 3.3, $p = 0.002$). These findings highlight the critical role of MEK in HCC progression and suggest its potential as a prognostic biomarker and therapeutic target.

Key words: institutional-normative factors, social norms covid-19, personal protective behaviour, mazandaran province

Introduction

With the rapid development of blood transfusion technology, automatic blood group analyzer based on MGT has been widely used in blood group serological detection, almost replacing the traditional blood group serological technology such as saline method, polybrene method and antiglobulin method. MGT has high sensitivity and specificity in crossmatching [1], and compared with polybrene method, MGT can significantly improve the detection rate of unexpected antibodies [2]. Wang Wenting et al reported[3] that 164,838 cases of cross-matching blood by MGT were negative, and 35 cases of positive blood were detected by cross-matching by saline method and polybrene method. The reason was analyzed that the laboratory over-relied on a single MGT for cross-matching and missed the detection of saline-reactive antibodies, but the report did not find unexpected anti-E missed during cross-matching by MGT. There were no reports of anti-E missed by the MGT when searching the literature on the "China National Knowledge Network", but there were more reports of anti-E detected by the MGT after the missed detection by the polybrene method [4], and the detection rate of anti-E by the MGT (37 cases) was higher than that by the polybrene method (20 cases) [5]. In the routine work of our laboratory, we found that there are two serological reaction characteristics of anti-E from human sources, one is missed by MGT and detected by polybrene method, and the other is detected by MGT and polybrene method. We speculated that there might be two kinds of anti-E immunoglobulins with high and low charges, and designed low ion and strong ion reaction system to verify the experiment, as reported below.

1. Materials and methods

1.1 General Information Case 1: The patient was a 77-year-old female with 3 pregnancies and 2 pregnancies and a history of transfusion. She developed fever and adverse transfusion reactions during transfusion of white suspended RBC. She was clinically diagnosed with hemorrhagic anemia, RBC:1.47×1012/L, Hb 47g/L, hematocrit 14.8%, blood group:O, RhD(+), and applied for transfusion of washed RBC. Case 2: A 32-year-old female patient with 4 pregnancies and 2 births, unknown transfusion history, clinically diagnosed hemolytic anemia, applied for infusion of washed RBC 4U.

1.2 Specific identification: Anti-E by MGT or polybrene method, unexpected antibodies were identified as anti-E, no autoantibodies or other unexpected antibodies, no lipids, no hemolysis.

1.3 Instruments and reagents: Diana microcolumn gel card incubator and special centrifuge, KA-2200 Kubota centrifuge. MGT provided by Diana Company; Polybrene reagents, screening cells, Pannel cells, monoclonal anti-A, anti-B blood group reagents, monoclonal anti-E are provided by Shanghai Blood Biomedicine Co., LTD. Pannel cell 2 was produced in Hungary (Lot: 732209); IgG monoclonal anti-D,IgG monoclonal anti-AB, and anti-globulin reagents are manufactured by DIAGNOSTICS SCOTLAND. ABO reverse reagent for RBC was made by our laboratory.

1.2 Methods

1.2.1 Blood group ABO/RhD blood group was performed using the saline test.

1.2.2 Screening and identification of unexpected antibodies The routine method is to first detect IgM unexpected antibodies by saline method, and then to screen IgG unexpected antibodies by MGT. If the unexpected antibodies are positive, Pannel cells are used for antibody specific identification. MGT and Polybrene method were used for screening and identification of IgG unexpected antibodies.

1.2.3 Cross-matching: Primary and secondary cross matching, the saline test method was used, and then the MGT and the Polybrene method were used for cross-matching.

1.2.4 Comparison between low ion reaction system and high ion reaction system

RhE positive RBC were selected, washed with 0.9%NaCl for 3 times, hematocrit was taken and Liss solution was used to prepare 4% and 1% RBC suspensions as low ion salt suspension RBC, and 0.9% NaCl was used to prepare 4% and 1% RBC suspensions as high ion salt suspension RBC. Human anti-E was diluted 1:4 with Liss solution and 0.9%NaCl, respectively. The suspended RBC and diluted human anti-E were detected by MGT and Polybrene method.

2.Results

2.1 Blood group identification ABO group: O, RhD(+).

2.2 Unexpected Antibody screening The sample 1 was negative in saline medium and MGT, and strongly positive "3+" in Polybrene method. Sample 2 did not agglutinate in saline medium, and the agglutination strength of cells screened by MGT was "2+", which was significantly higher than that by Polybrene method (see Table 1).

Methods	Samples 1				Samples 2			
	O1	O2	O3	Control	O1	O2	O3	Control
saline medium	-	-	-	-	-	-	-	-
MGT	-	-	-	-	2+	-	2+	-
Polybrene	3+	-	3+	-	±	-	±	-

Table 1: Results of unexpected antibody screening by three serological methods

2.3 Unexpected Antibody identification In sample 1, unexpected antibody identification was performed by MGT, and cells I to X were negative (see Figure 1); Unexpected antibody identification was performed by Polybrene method, and cells II, IV and IX were positive (see Figure 2), and the reaction pattern was completely consistent with that of anti-E. The anti-E in sample 1 was missed by MGT. Sample 2 was unexpectedly identified by MGT. Cells II, VI, VII, VIII and X of the Pannel cells were positive (see Figure 3). Cells I - X of the Pannel cells were negative by Polybrene method. The RBC E antigen of the two patients was identified by monoclonal anti-E, and the presence of anti-E in the plasma of the patients was determined with titers of 1:8 and 1:4, respectively.

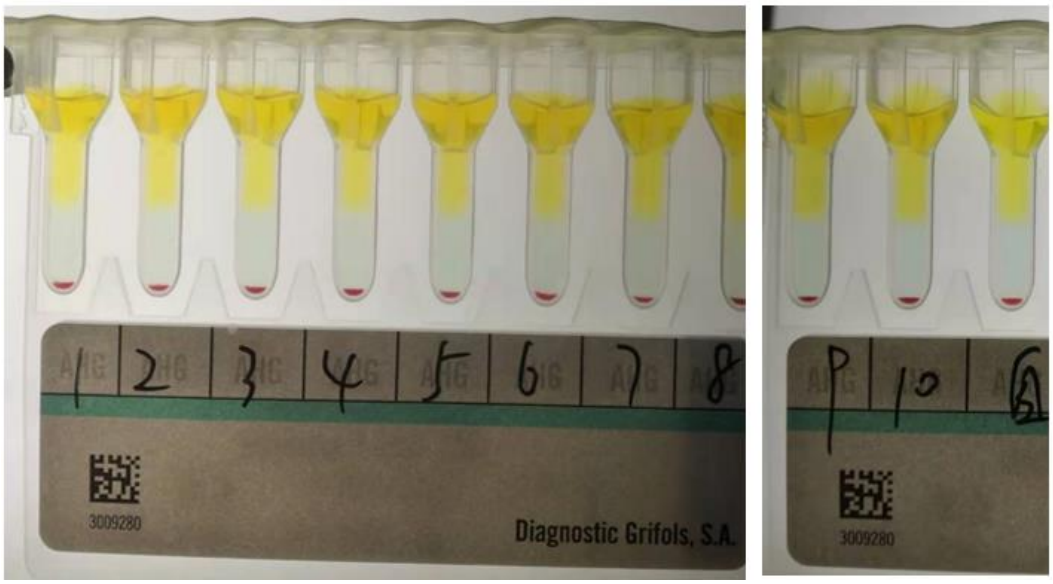

Figure 1: Unexpected antibody identification by MGT

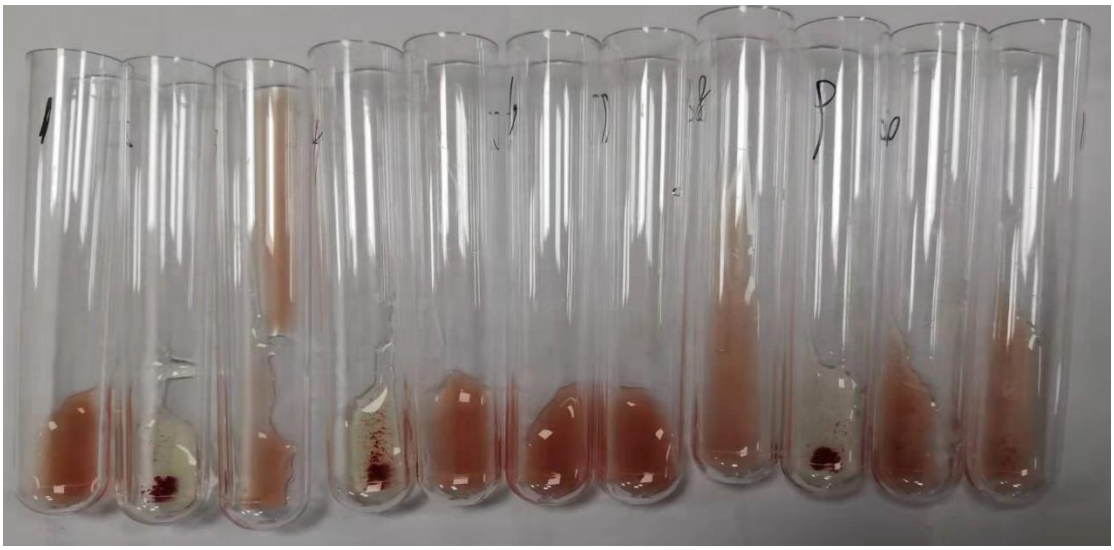

Figure 2: Unexpected antibody identification by Polybrene method

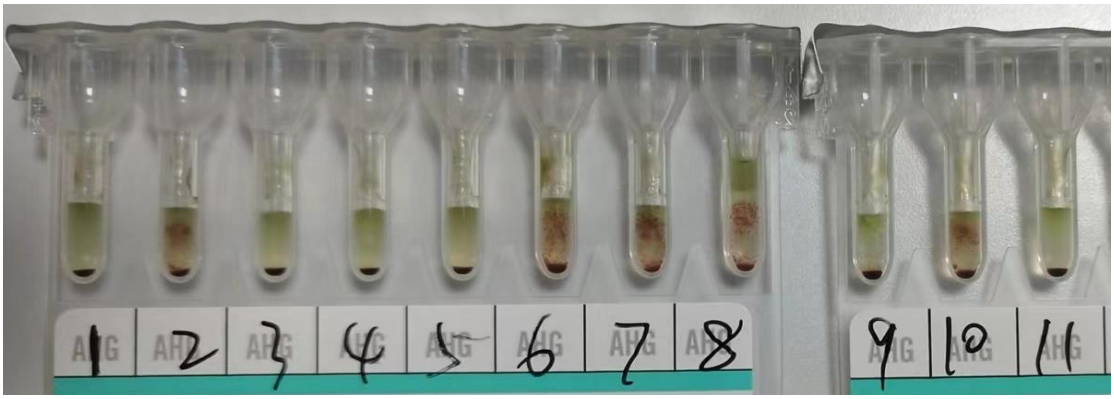

Figure 3: Results of unexpected antibody identification in sample 2

2.4 Cross matching O, RhD(+)Ec (-) RBC were selected for cross matching, and there was no agglutination or hemolysis in saline medium, MGT and polybrene method. RBC from Rh Ec(+) simulated donors were selected to exhibit agglutination reaction on the main cross-matching with the polybrene (see Table 2).

	ABO	Rh	Sample 1 main cross matching			Sample 2 main cross matching		
			N.S	MGT	Polybrene	N.S	MGT	Polybrene
Donor 1	O	D (+),Ec（-）	-	-	-	-	-	-
Donor 2	O	D (+),Ec（-）	-	-	-	-	-	-
Simulated donor	O	D(+),Ec（+）	-	-	3+	-	2+	±

Table 2: Results of cross-matching between donor and simulated donor

2.5 Comparison between Liss liquid (low ion) and 0.9% NaCl (strong ion) reaction system. The concentration of 4% Liss liquid suspended RBC and 0.9% NaCl suspended RBC were reacted with 1:4 diluted plasma containing anti-E, respectively. Sample 1 was negative by MGT and positive by polybrene method. Sample 2 was positive by MGT and stronger than polybrene method, especially negative in 0.9% saline medium after dilution (see Table 3).

Reaction system	Sample 1		Sample 2	
	MGT	Polybrene	MGT	Polybrene
0.9% NaCl suspended red blood cells + human anti-E serum	-	2+	2+	+
Liss liquid suspension Red Blood cells +Liss diluted plasma (1:4)	-	$2+^{s}$	3+	1+
0.9% NaCl suspended red blood cells +0.9% NaCl diluted plasma (1:4)	-	$+^{s}$	1+	-

Table 3: Comparison of Liss liquid (low ion) and 0.9% NaCl (strong ion) reaction system detection

3.Discussion

The commonly methods for detecting IgG antibodiesinclude polybere method, antiglobulin method, MGT and enzyme medium method. The saline method is mainly used to detect IgM antibodies, which is an indispensable step in cross-matching. This method can find IgM unexpected antibodies, Group errors or determine whether hemolysis occurs, but cannot detect IgG unexpected antibodies. The process of polybere method is quick, simple and manual operation, but it is not sensitive to the antibodies of Kell group system, and K antigen in Chinese is almost 100% negative, so this method is widely used in primary medical institutions. Antiglobulin method is recognized as the "gold standard", but its operation time is long, there are many steps, many influencing factors, and it needs manual operation. Based on the gel filtration technology and the specific reaction principle of immunochemical antigen and antibody, the MGT controls the size of the gel gap by adjusting the concentration of the gel, so that the gap can only allow free RBC to pass through, so that free RBC and agglutinated RBC can be separated. This method has high sensitivity, simple operation, clear, and easy automation. And there is no need to worry about the false negative caused by the neutralization of anti-globulin antibodies, so this method has been widely used, and even will completely replace the saline method, polybere method and other methods.

Unexpected antibodies are a major cause of cross mismatch, most commonly in the Rh system. It was reported that the cross mismatch caused by unexpected antibodies accounted for 80.9% of the total cross mismatch [6], among which 25.9% (14/54) was caused by anti-E in the Rh group system [7], and there were more reports of hemolytic transfusion reactions caused by anti-E [8-13]. It is generally believed that the sensitivity of MGT to detect unexpected antibodies is higher than that of the ploybere method, but there are few reports of missing anti-E detection by MGT in China. Wang Wenting et al. [3] reported that 164,838 cases of cross-matching blood with MGT were detected by MGT, and no missed anti-E was found, and 35 cases of missed antibodies were all IgM unexpected antibodies. The anti-E found in this study was missed in antibody screening and antibody identification by MGT, but showed obvious positive reaction by polybere method. There was no positive reaction when Ec (+) RBC were cross-matched by MGT, but there was agglutination reaction when the main side was cross-matched by polybere method. In this case, if only the MGT is used for cross-matching, the RBC of E (+) may be mistakenly sent to the clinic, which may lead to delayed hemolytic transfusion adverse reactions. It has been reported that the sensitivity and specificity of polybere method used in cross-matching and screening of incomplete antibodies are 1-250 times higher than other media [14,15]. Polybere is more sensitive in the determination of most antibodies (such as Rh system antibodies, Kidd system) than enzymatic method and traditional antiglobulin method [16]. The anti-E sensitivity of sample 1 was better than that of the MGT. On the contrary, the reaction strength of sample 2 was stronger than that of the MGT. When the original plasma was diluted to 1:4, the polybere rule was missed. It is speculated that the amount of charge carried by the anti-E immunoglobulins produced by different individuals in the RBC is different, thus affecting the affinity between the anti-E immunoglobulins and the corresponding antigens. In the strong ionic medium, the anti-E immunoglobulins carrying more negative charges have a strong charge rejection effect on the RBC, so the antigen-antibody reaction cannot be well completed in the low ionic medium. The amount of charge of immunoglobulin is reduced, the rejection is weakened, but it is more prone to antigen-antibody reaction, which shows a positive reaction in the polybere method, and a negative reaction in the MGT. However, the charge repulsion of RBC with low charge of anti-E immunoglobulins and corresponding antigen is weak, and they are more sensitive in MGT. Experiments confirmed that the amount of charge carried by anti-E immunoglobulin and RBC was reduced in the low-ion solution system, and the decrease of charge repulsion made it easier for the RBC antigen and immunoglobulin antibody to bind to the immune antigen and antibody, showing the enhanced reaction in the low-ion salt solution, which also explained the reason for the missed detection by MGT method in the high-ion reaction system. In this report, it is speculated that there are two types of anti-E immunoglobulins derived from human sources, one of which carries a high negative charge, such as the anti-E derived from sample 1, and the other carries a weak negative charge, such as sample 2. The detection sensitivity of MGT is higher than that of polybere, which is consistent with most literature reports that such antibodies are easily missed by polybere [4,5]. Therefore, there is no single method that can detect all antibodies at the same time, and cross-matching methods with different sensitivity and specificity should be selected for different patients or antibodies[17]. At present, there is a lack of direct evidence for this speculation, such as detecting the amount of charge carried by immunoglobulins.

In summary, this study confirmed that there are two types of human anti-E, one missed by MGT and the other missed by polybere method. It is speculated that these two types of human immunoglobulins have the same immune reactivity, but the amount of charge they carry is different. Different media may have differences in different blood group systems or different specific antibodies, and a single method may cause unexpected antibody missed detection. It is advisable to use more than two media methods, and take advantage of the advantages of the two methods, such as low-ion medium micro-gel method, to better ensure the safety of clinic.

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