

Is Wait and Watch Policy More Appropriate in Newborn Grouping?

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Abstract

ABO grouping is one of the most simple but important tests in the field of transfusion medicine. Because ABO antibodies in cord blood are nearly always of maternal origin, ABO testing on newborns relies entirely on red cell typing. In this case we performed serological work up to determine the cause of weak reactivity and mixed-field agglutination in newborn baby's sample. Even though the immunohematological results mimicked that of the 'A₃' subgroup initially, later confirmed to be weak 'A' antigen. So it may be wise to wait and follow up the case rather than diagnosing the presence of subgroups during neonatal blood grouping.

INTRODUCTION

Blood grouping, even though is a simple laboratory test, has got great importance in the field of transfusion medicine. Discrepancy is a common problem faced during routine blood grouping. Resolving the discrepancy as well as interpreting the correct blood group are challenging in many cases. Blood grouping is one of the commonly done tests in cord blood of newborn. There are various issues involved in blood grouping of newborns like false positive results in cord blood if washed improperly and less relevance of reverse grouping due to maternal antibodies. In the present case we performed the serological work up to trace reason for weak and mixed-field reaction while cell typing and found that even though the results mimic the subgroup it is due to the presence of weak antigen.

CASE REPORT

Cord blood samples were sent to the blood bank for the routine blood grouping of term new born twin babies (within an hour of birth). By following the departmental standard operating procedure for blood grouping in cord blood, test was carried out. As shown in the table 1 the second of twin was showing group O, whereas in the first of twin, the group was not interpretable. The reaction with antisera A showed 2+ reaction and on microscopic examination there were free cells along with few agglutinates giving mixed field appearance. Rouleaux formation was ruled out by a negative control and saline replacement technique. Since the reverse grouping was not done in this baby as per accepted practice, there was no clue about the antibodies in the serum.

Figure 1

Table 1: Initial testing results.

Cord samples	Anti A	Anti B	Anti D	Anti AB	Control(cord cells+ saline)	Interpretation
Twin 1	2+ mf	4+	4+	4+	Negative	?
Twin 2	Negative	Negative	Negative	Negative	Negative	O RhD positive

As the sampling or technical errors are the one of the common causes for blood group discrepancies, repeat sample (venous) was requested to rule out any of these errors. Repeat testing showed the same results. Grouping was done using standard tube technique using antisera from Tulip diagnostics (Verna, Goa-India) as well as using fully automated technique using AUTO/VUE ABD cards by Ortho-Clinical Diagnostics (a Johnson & Johnson company, UK).

Quality control of the automated grouping technique was done by using samples, with known blood groups and the expected results were obtained. Reagents quality control of anti A, anti B, anti D and Anti AB was done by physical examination, titration, specificity and avidity testing. On physical examination reagents were clear and no precipitate. Titer was >256 and thus meeting the drug controllers criteria [1]. All reagents passed in specificity testing.(Anti A reacted only with A₁, A₂ cells not with B group or O group cells, similarly Anti B showed reaction with with B cells and not with A or O group cells).Avidity was <4 seconds for Anti A, Anti B, Anti AB and 5.8 seconds for Anti D.

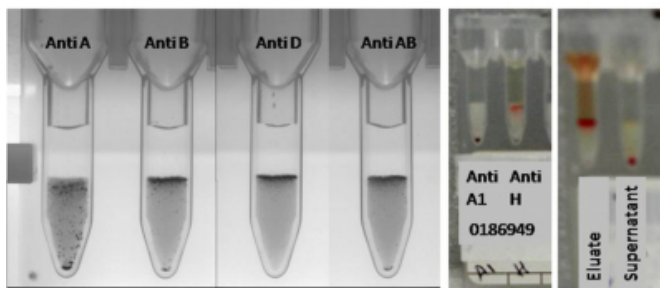
We proceeded with testing the cells with anti A₁ lectin, which gave a negative reaction and Anti H lectin showed strong positive result (4+). To prove the presence of 'A'

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antigen, adsorption with polyclonal anti A was done as per protocol [2]. After 1 hour of incubation with the anti A at 4°C, cells were washed eight times saving the supernatant of final wash. To dissociate the adsorbed antibodies heat elution was done. On simultaneous testing of the supernatant from final wash and the eluate, both gave negative results with A cells by using immediate spin technique at room temperature. Indirect antiglobulin test was done after 15 minutes of incubation at 37°C. It showed a strong 4+ reaction with eluate and absence of agglutination with the supernatant, thus proving the presence of A antigen (figure 1).

Figure 2

Figure 1: Image showing the results of serological testing



Since one of the differential diagnoses was subgroup of A (A3B) we analyzed the family. Family studies included parental blood grouping along with testing for the subgroups of A. As shown in table 2, father’s group was ‘B’ and mother’s group was ‘A’ also giving 4+ reaction with anti A1 lectin. Pediatrician in charge of the baby and the parents were informed regarding the presence of weak ‘A’ antigen along with B antigen and advised repeat grouping after 6 months of age. While follow up the strength of reaction with antisera A increased and finally it was reported as group AB (Table 2).

Figure 3

Table 2: Further serologic testing

	Anti A	Anti B	Anti D	Anti AB	Anti H	Anti A1	A cell	B cell	O cell	Inference
Twin 1 (venous sample)	2+/mf	4+	4+	4+	4+	0	NA	NA	NA	?
Mother	4+	0	4+	4+	4+	4+	0	4+	0	A RhD positive B RhD positive
Father	0	4+	4+	4+	4+	0	4+	0	0	B RhD positive
Twin 1 (4 months)	3+	4+	4+	4+	4+	1+	NA	NA	NA	AB RhD positive

DISCUSSION

ABO grouping is one of the most simple but important test in every transfusion setup. It consists of both cell grouping and a serum grouping; interpretation of these two tests together supports a common conclusion, and ABO group is

confirmed. Because ABO antibodies in cord serum are nearly always of maternal origin, ABO testing on newborns relies entirely on red cell typing.

In this case we performed serological work up to determine the cause of weak reactivity and mixed-field agglutination in newborn baby’s sample. As there are various reasons which may lead to the decrease in strength of the agglutination reaction a step by step approach was done to delineate the cause.

The suspected causes of weak and mixed-field reaction in this case were

- Sample/ reagent related problems or Technical errors
- Neonatal patient- weak antigen giving the weak reaction
- Subgroup of A-giving weak and mixed field reaction
- B(A) phenotype (because the anti A reagent by Ortho diagnostics had MHO4 clones)

To rule out the technical errors or sample related problem the repeat testing was done using the venous sample of the baby. And quality control of the reagents and tests were meeting the required criteria.

The blood grouping reagents which were used for the testing (by Ortho-Clinical Diagnostics, UK) contain anti A murine (IgM) monoclonal antibody blend (clones MHO4 and 3D3). According to Beck et al, red cells of some group B individuals are agglutinated by a licensed anti A reagent that contains a particular murine monoclonal antibody, MHO4 and the designation B(A) was given to this blood group phenotype[3]. B(A) red cells can show varying reactivity with anti-A. Testing with an anti A without the MHO4 clone (by Tulip Diagnostics and by Diagast Laboratories) excluded the possibility of B(A) phenotype by giving the same result as the previous reagent.

Here, newborn patient’s serologic findings are consistent with the expected findings of an A3 adult sample. Reaction with anti A was weak and mixed-field, and negative with anti A1 lectin. Adsorption elution showed the presence of A antigen. There is both a qualitative and quantitative difference between subgroups of A. Generally classification of weak A subgroups is based on the following details[4]:

- Degree of red cell agglutination by anti-A and anti A1
- Degree of red cell agglutination by anti AB
- Degree of red cell agglutination by anti-H (ulex europaeus)
- Presence or absence of anti A1 in the serum
- Presence of A and H substances in the saliva of secretors
- Adsorption and elution studies
- Family (pedigree) studies

MOLECULAR TECHNIQUES

Results of serological tests were similar to the adult A3 subgroup, but blood group study of parent's rules out the possibility of inherited A3 subgroup. Molecular studies could have been done to detect the transferase enzymes responsible for A, B, and O phenotypes and to distinguish the mutations which results in many of the subgroups, including the A3 phenotype. But these molecular studies were not available as in most of the other centers in developing countries.

It is well known that although 'A' and 'B' antigens can be detected on the red cells of 5 to 6 week old embryo, A and B antigens are not fully developed at birth[4]. Because ABO antigens are not fully developed at birth, the red cells of newborn who are genetically group A1 may not react or only weakly with anti A1 lectin [5]. Mixed -field appearance of agglutination with anti A may be related to poorly developed antigen expression rather than genetically inherited A3 subgroup.

The red cells of new born have approximately one third the number of A and B sites as adult cells. A1 adults have approximately 0.8×10^6 antigen sites per red cell. A2 adults have approximately 0.24×10^6 antigen sites per cell, which is comparable to the number of antigen sites per cell seen in A1 newborns ($0.25-0.37 \times 10^6$). A3 adults have approximately 0.035×10^6 antigen sites. This comparison of

number of antigen sites becomes all the more complex because this infant also has B antigen [6]. The differences between the B blood cells of the newborn and the B blood cells of the adult are similar to those which have been found to exist within the blood group A. However, these differences are less impressive from the quantitative point of view than they are in the case of blood cells of group A [7]. Since there are less than 50% of the adult antigen sites are present at birth it may lead to an inability to differentiate adequately subgroups on newborn cells [8]. So whenever there is a weak expression of antigen in newborn, implementation of routine practice to follow up of the case will be more cost effective than immediate further workup for the sub grouping by serological or molecular testing.

CONCLUSION

In our country most of the transfusion centers and blood banks may not have the facility for the complete immunohaematology work up and molecular diagnostic facilities for the differentiation of sub grouping or the weakly expressed antigens. So even though there are overlapping results in serology, it may be wise to wait and follow up the case rather than diagnosing the presence of subgroups during neonatal blood grouping.

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