Antibodies detected in samples from 21,730 pregnant women

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Although anti-D is still the main cause of HDN, many other antibodies have been implicated. From September 1995 to April 2000, screening for RBC antibodies was performed on samples from 21,730 pregnant women regardless of RhD type. Standard tube and gel methods were used. Anti-D was identified in 254 samples; other antibody specificities were detected in 376 samples, for a total of 630 antibodies. For this study, 522 antibodies were considered clinically significant. The incidence of potentially clinically significant antibodies was 2.4 percent. The majority belonged to the Rh system, followed by anti-M, -Fy^a, -S, -Jk^a, and -Jk^b. Among antibodies of no clinical significance, the most frequent were anti-H, -Le^a, and -P_1. ***Immunohematology* 2003;19:89–92.**

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Prenatal immunohematologic care of pregnant women requires the investigation of unexpected RBC antibodies in their sera during pregnancy. When RBC antibody screening is positive, it is necessary to determine specificity of the antibody, its clinical importance, and the ability to cross the placenta and cause HDN.

Anti-D is still the main cause of HDN, despite the use of systematic RhD immunoprophylaxis since 1968 in many developed countries. The incidence of anti-D decreased from 17 percent to 1.5 percent after the administration of postpartum prophylaxis, and from 1.5 percent to 0.6 percent after antepartum Rh immunoprophylaxis.[1] In Yugoslavia, the incidence of alloimmunization to D is still high despite postpartum RhD immunoprophylaxis. According to several authors, the incidence of anti-D in our country, where RhD immunoprophylaxis is administered only postpartum, is between 1.28 percent and 1.68 percent.[2,3]

According to published data, the incidence of clinically significant antibodies during pregnancy in Croatia is approximately 1 percent (64.8% anti-D), 0.58 percent in Tyrol (54% anti-D), 0.82 percent in Salzburg (48% anti-D), and 0.24 percent in Sweden (32% anti-D).[4–6]

The aim of our study was to determine the current incidence of non-anti-D antibodies in both D– and D+ pregnant women in our country.

Materials and Methods

From September 1995 to April 2000, RBC antibody screening was performed on blood samples from 21,730 D+ and D– pregnant women at the Laboratory for Antenatal Screening of the National Blood Transfusion Institute (NBTI). Transfusion and previous pregnancy histories were unobtainable in most cases. RBC antibody screening was done at room temperature, using enzyme-treated RBCs (papain, Merck), and by the IAT using a tube method. Enzyme-treated and non-treated group O reagent RBCs were prepared in our Institute according to standard procedures. Suspect and positive results of antibody screening were checked by the gel method (DiaMed ID, Cressier sur Morat, Switzerland), with commercially prepared reagent RBCs from the same manufacturer. Antibody identification was performed by either the tube or the gel method. Commercially prepared RBC panels were provided by the NBTI and by DiaMed. Polyspecific and monospecific anti-human globulin (AHG) test reagents were products commercially prepared in our Institute and by DiaMed. Antibody titration was done, whenever possible, using a manual method by IAT using monospecific anti-IgG.[7–10]

Results

The incidence of anti-D found in the 21,730 samples in our study was 1.16 percent, which is similar to the incidence of anti-D found in a previous investigation (1.36%).[2] Anti-D was demonstrated in 254 (40.0%) of the 630 antibody-positive samples (Table 1), however, 54 were attributed to "passive" anti-D postpartum prophylaxis.

Most of the clinically significant non-anti-D antibodies belonged to the Rh system (77%). The most common antibody in this group was anti-C (23%), which was, with one exception, always identified with anti-D (Table 1).

There were 67 anti-E (11%) in the sera of both D+ and D– pregnant women. There were 17 anti-c (2.7%) and 2 anti-C^w (0.3%); 12 of 17 anti-c and the majority of anti-C were confirmed using enzyme-treated RBCs. The remaining five anti-c antibodies were identified by IAT, with antibody titers between 8 and 16. All 67 anti-E and the two anti-C^w were identified only using enzyme-treated RBCs (Table 1).

Table 1. Specificity and percentage of 485 clinically significant Rh antibodies detected in 630 positive blood samples from pregnant women

Antibody	Number detected	% detected
D	254	40.0
C	145	23.0
E	67	11.0
c	17	2.7
C^w	2	0.3
Total	485	77.0

Anti-M was identified in 23 samples (3.7%). All were identified at room temperature. Three were also present in the IAT using anti-IgG AHG.

Anti-Fy^a was identified in six cases (0.9%) in our study; the antibodies of lowest incidence were four anti-S (0.6%), three anti-Jk^a (0.5%), and one anti-Jk^b (0.2%) (Table 2). All of these antibodies were detected by IAT.

Table 2. Specificity and percentage of 37 clinically significant non-Rh antibodies detected in 630 positive blood samples from pregnant women

Antibody	Number detected	% detected
M	23	3.7
Fy^a	6	0.9
S	4	0.6
Jk^a	3	0.5
Jk^b	1	0.2
Total	37	5.9

Among the antibodies without clinical significance, the most frequent were 49 anti-H (7.8%), which were identified using enzyme-treated RBCs, mostly at room temperature. Anti-Le^a was present in 30 samples (4.7%), anti-Le^b in 17 samples (2.7%), and anti-Le^a+Le^b in 3 samples (0.5%). In most of the investigated cases, Lewis antibodies were demonstrated using enzyme-treated RBCs and, less commonly, by IAT. They usually caused hemolysis using enzyme-treated RBCs.

Table 3. Specificity and percentage of 108 non-clinically significant antibodies detected in 630 positive blood samples from pregnant women

Antibody	Number detected	% detected
H	49	7.8
Le^a	30	4.7
Le^b	17	2.7
P_1	9	1.4
Le^a and Le^b	3	0.5
Total	108	17.1

Nine examples of anti-P_1 (1.4%) were detected at room temperature and using enzyme-treated RBCs (Table 3).

Due to the number of samples (21,730) sent for testing from various sources over a period of 5 years, it was not feasible in most cases to obtain individual pregnancy and transfusion histories.

Discussion and Comment

Anti-D can cause both a moderate and a severe form of HDN. The incidence of anti-D alloimmunization in D– women without the administration of prophylactic anti-D during pregnancy is usually noted at the end of a second pregnancy, with an incidence of 8 to 10 percent, and after the fourth or fifth pregnancy, at 50 percent.[1,4,11]

The most frequent and potentially significant non-anti-D antibody in our study was anti-C. The incidence among both D+ and D– pregnant women was 23 percent. Bowell found an incidence of 14 percent in D+ pregnant women.[18] HDN caused by anti-C is usually mild, as the C antigen has weak immunogenicity.[1,11–13]

Anti-E was found in 11 percent of our cases. Anti-E can be a naturally occurring IgM antibody, as it was in most of our cases. IgG anti-E can be found in the sera of pregnant women with a history of previous transfusions and pregnancies. This immune form of anti-E is able to cause mild or moderate HDN.[1,4,11,14]

The incidence of anti-c in our investigation was 2.7 percent, which is similar to Bowell's results for pregnant women.[15] In most women, alloimmunization to the c antigen is found after multiple pregnancies, transfusion of c+ RBCs, or both. Mild and moderate cases of HDN usually appear when the titer of anti-c is higher than 8 by IAT.[4,15]

Anti-C^w in our study had an incidence of 0.3 percent. This antibody can sometimes cause mild HDN.[1,4]

Anti-M had an incidence of 3.7 percent in our study, which correlates with results in similar investigations. These antibodies are usually naturally occurring IgM + IgG antibodies. A clinically significant IgG anti-M has been reported as a cause of hydrops fetalis.[1,6] The incidence of clinically significant IgG anti-M is 0.1 percent.[16,17]

Anti-S seldom causes HDN.[1,4] In our study, the incidence of this antibody was 0.6 percent.

According to published data, anti-Fy^a can be found in 33 percent of Fy(a–) persons transfused with Fy(a+) RBCs. Anti-Fy^a rarely causes HDN, but some of the described cases were fatal.[1] There were six examples of anti-Fy^a in our study, with an incidence of 0.9 percent.

Anti-Jk^a and -Jk^b (0.5% and 0.2%, respectively), complement binding antibodies, were rarely found in our investigation. Published data show that they seldom cause HDN, regardless of antibody titer.[1] So far there is no explanation for that, although complement is not fully developed during fetal life nor right after delivery.[4]

Anti-K was not seen in our investigation. The incidence of the K antigen in Caucasians is 9 percent and, after the D antigen, the K antigen is the most immunogenic. HDN caused by anti-K can be severe.[1] There is evidence that anti-K can recognize K antigens expressed at an early stage of erythroid development in the fetal liver and can cause anemia by suppressing erythropoiesis.[1,18,19]

Anti-H, -Le^a, -Le^b, and -P_1 have little or no clinical significance even though they can often be found in the sera of pregnant women. It is well known that expression of Lewis antigens is reduced during pregnancy. The explanation is in the slight decrease of Le glycolipid in the plasma during pregnancy and in the increased ratio of lipoprotein to RBC mass that occurs in pregnant women. There is not an exact explanation for the higher appearance of anti-H and -P_1 during pregnancy. The incidence of these antibodies in our study was 7.7 percent and 1.4 percent, respectively.

Guidelines for prenatal immunohematology in most countries suggest testing for unexpected antibodies at the initial visit, regardless of the woman's D type. Third trimester testing is recommended for D– women and for both D– and D+ women when there is a history of previously detected clinically significant antibodies, blood transfusions, or complicated deliveries.[20]

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