The Significance of Imunohematology Research in Relation to Menagement of Hemolitical Diseases of the Newborn in Republic of Macedonia

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Abstract

AIM: Prompt discovery of allosensibilisation to RBC's antigens during pregnancy and successful management of HDFN in Republic of Macedonia, in order to decrease morbidity and mortality of the fetus and the newborn.

MATERIALS AND METHODS: The study comprises in total 23,800 patients, 14,858 pregnant women and 8,842 newborn babies.

RESULTS: The screening and identification of anti RBC's antibodies detected in total 216 alloantibodies, out of which 81% (175) had a clinical significance. Out of the above mentioned 164 alloantibodies (65.9%) belong to the Rh system. The most often reason for a severe hemolytic disease is the anti-D antibody. The HDFN symptoms of mild and moderate degree demonstrated 32.5%, and 18.9% had symptoms of severe fetal suffering, and almost half of them (48%) were with or with mild HDFN and had no need of therapy. In 15% it was about alloantibodies of other Rg antigens: anti-C, anti-E and anti-c, at which in most cases there were no signs of HDFN, or it showed weak symptoms (89%), just one case of anti-c ended with intrauterine death.

CONCLUSIONS: Anti D antibody represents the most often reason for severe HDFN and displays a need of intrauterine transfusion and exsangvino transfusion. Anti-c is the only antibody that demonstrated the same potential for severe HBN as the anti-D. The most often reason for alloimmunisation of the mother is the lack of RhIG prophylaxis (97.8): postnatal, antenatal and in case of possible sensible conditions during pregnancy. Thus, there is a need and an outmost importance of elaboration and adoption of the National programe for RhIG prophylaxis in Republic of Macedonia.

Introduction

The hemolytic disease of the fetus and the newborn (HDFN) is a clinical syndrome at which the basic patho-physiological disorder represents a hemolytic anemia of the fetus or the newborn. HDFN starts during the intrauterine life, and the cause of the disease is the presence of IgG antibodies that appear as a result of aloimunization of the mother towards the Red Blood Cells (RBC) antigens of the fetus, inherited from the father, and not present in the mother.

It is directly dependant of the development of RBC's antigens on the surface of fetal RBC. The RBC's membrane of the newborn does not comprise in full development of all antigens that are present in adult's RBC. The antigens whose specific characteristics are determined by imunomodulator sugar components (ABH, Lewis, P, Sd^a) are not well developed immediately after birth, compared to protein antigens (Rh, MNS, Duffy, Kell, Kid), which are completely present. A and B antigens can be proven at an embryo as soon as the 5-6 gestation week, but after birth their expression is only at 50%, because of lack of the H-substance as a substrat on which the enzymes work for creation of additional imunodominant sugar components. I and Lewis antigens are fully developed at the 18th month of the newborn after birth [1]. In comparison, the D antigen can be tested/proven as soon as the 38th gestation day, and it is well developed after birth [2]. RBC's

antigens at a newborn [1] and the level of their presence can be divided into three groups:

- RBC's antigens that are well developed at the newborns' RBC: Diego, Dombrock, Duffy, En[a], Gerbrich, Kell, Kidd, MNSs, Rh, Sciannia, Yt[b]

- RBC's antigens that exist on the newborns' RBC's membrane, but are less expressed compared to adult's erythrocytes: ABH, Lutheran, P, Xga

- RBC's antigens that are much less expressed or completely absent at the newborns' RBC: Chido, Rogers, I, Lewis, Sd[a], Vel, Yt[a]

The diseases' pahto-physiology is connected to the transfer of antibodies from the mother to the fetus which is done exclusively through the placenta. At the same time, the placenta is an imunologic barrier where the trophoblast rich with sialin acid, besides the IgG antibodies, lets in and out only certain cell elements in both directions.

RBC, Platelet and WBC of the throphoblast and imunoglobulines pass from the fetus towards the mother, and from the mother to the fetus pass only the mother's RBC, Platelet and IgG. The only immunoglobulin transfer is the one of the IgG antibodies that get tied to the Fc receptor of the placenta's plasma membrane as an active transport that goes on in only one direction from the mother to the fetus and never vice versa [3].

It should be especially emphasized that the fagocites of the fetus for IgG anti-bodies, there are FcyRI receptors that are responsible for hemolysis of RBC coated with antibodies [4, 5]. The immunization of the mother will take 0.1ml of fetal RBC. The frequency of transplacenta hemorrhage is directly dependant on the gestation week and increases further on during pregnancy.

There are more factors known that influence the destruction of IgG sensibilized RBC, determining the strength of HDFN: special characteristics of alloantibodies, concentration of alloantibodies, IgG glycolization, IgG subclasses; antigen density of erythrocytes, structure, tissue (placenta) distribution; the function of the fetal spleen, FcR polymorphism, inhibiting antibodies, fetal abgar. The most important information that demonstrates potential clinic significance of anti RBC's alloantibodies at HBN is their distinctiveness.

Accordingly, one of the reasons for habitual abortions, fetal death, as well as premature birth with symptoms of severe hemolitical disease at newborns is the alloimmunization of the mother to RBC's antigens.

One of the primary goals of immuno-hemathological testing as a part of antenatal testing to HDFN is to provide clinically valid information to the cooperation team (gynecologist – obstetrician), in the true sense of the word "cost effect". The validity of information comprises determination of need and exact time to begin with invasive monitoring of HDFN – amniocentesis, cordocentesis – with a risk of transplacental hemorrhage, exacerbation of alloimmunization and increase of fetal anemia, and a need of Intra Uterine Transfusion. Thus, immune-hematological testing can greatly contribute in avoidance of this risk.

Table 1: A probability for serious HDFN associated to anti-RBC antibodies (A. F. Eder) [18].

Blood type system	The biggest probability for severe	Rare cases of severe HBN	Most often associated to moderate severity	Do not cause HBN
MNS		M, S, s, U, Mi[a], Hut, Mur, Mt[a], Hil, M[v], Far, sD, MUT	M, S, s, U, Mt[a] Mit	N
Rh	D, c	C, E, f, Ce, C[w], C[x], E[w], G, Hr[o], Hr, Rh29, Go[a], Rh32, Be[a], Evans, Tar, Rh42, Sec, JAL, STEM	E, e, f, C[x], D[w], Rh29, Riv, LOCR	
Luteran			Lu[a](rare), Lu[b]	
Kell	K	k, Kp[a], Kp[b], Ku, Js[a], Js[b], Ul[a],K11, K22,	Ku, Js[a],K11	K23, K24
Lewis				Le[a], Le[b]
Dufy		Fy[a]	Fy[b](rare), Fy3(rare)	
Kidd		Jk[a]	Jk[b](rare), Jk3	
Others		Di[a], Wr[a], Rd, Co[a], Co3, PP1P[k], Vel, MAM Bi, Kg, JONES, HJK, REIT	Di[b], Sc3, Co[b], Ge2, Ge3, Ls[a], Lan, At[a], Jr[a], LFV, HOFM	P1, Wr[b], Yt[a], Yt[b], Sc1, Sc2, CH/RG CROM, KN, I, JMH, Jr[a], HLA: Bg[a], Bg[b], Bg[c]

Materials and Methods

The study comprises in total 23,800 patients, 14,858 pregnant women and 8,842 newborn babies, during a period of 8 years.

The immune-hematological testing for all pregnant women has been executed in the immune-hematology laboratory at the Institute for Transfusion Medicine in Republic of Macedonia. The tests have been executed in the serum and RBC of vein blood, without anticoagulant and/or EDTA, not older than 24 hours.

The immune-hematological testing for newborn babies has been executed of vein and umbilical blood, without anticoagulant and/or EDTA, not older than 24 hours.

The immune-hematological testing that are used in this study:

- blood type typization of RBC's antigens;

- screening and irregular anti-RBC's antibodies (IAT, enzyme test and DAT).

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Testing that has been applied to patients with positive screening tests:

- identification of anti-bodies with commercial panels for all patients;

- elution of antibodies;

- titer of anti-bodies (salty, Coombs and enzymes' tests).

Statistic methods

During the process of research acquired data are statistically elaborated with the following statistic methods:

- Statistic series according all defined variables of interest, displayed in tables and graphs;

- Analysis of the structure of attributive statistic series has been done by coefficient of relations, proportions and rates;

- Testing of difference significance between two proportions has been done by the Students test of proportions.

Hemolytic disease of a newborn baby in correlation with immuno-hematological analysis

All blood samples taken from pregnant women, where immuno-hematological analysis demonstrated positive results, incited continuous immuno-hematological testing of the mother during pregnancy (every 2-3 weeks) and follow up of the fetal development on the Clinic for Gynecology and Obstetrics – Skopje, on the ward for pathology pregnancy. All newborn babies of sensibilized mothers were treated according a special monitoring for at least five days on the ward for pediatrics in CGO. In total 216 alloantibodies were discovered, out of which 164 belong to the Rh system. The results have shown that the most often reason for a severe hemolytic disease is anti-D antibody in 51.4 % of the total number of alloantibodies, or in 80.4% of the cases with Rh sensibilization. 32.5% of the sensibilizations related to this antigen showed symptoms of HDFN with moderate and serious range, and a need of phototherapy and/or exsangvino transfusion. In 18.9% were shown symptoms of severe fetal suffering, demonstrated by hidrops fetalis or fetal death.

Almost half (48%) of the D positive newborns from mothers that have a detected anti D antibodies were without or with a mild HBN and had no need for therapy.

The rest of the Rh anti erythrocyte antibodies that do not belong to anti-D and are most frequent are: anti-E and anti-C. Out of 8 patients anti-E only 1

needed therapy (phototerapy. They rarely give a strong demonstration of HBN.

Anti-C is the only antibody that showed the same potential for severe HBN as the anti-D with a fetal hidrops. When a presence of anti-C antibody is detected the possibility to cause HBN is higher (44%), than anti-E (12.5%), and in these cases most often an exsangvino transfusion or phototherapy is needed (33%), than at the anti-E (10%).

The comparison of sympthoms and therapy at newborns with immuno-hematology research of their mothers has been shown on the Table 2.

Table 2: Distribution of antierythrocyte antibodies and the severity of the clinical picture of HBN in Republic of Macedonia.

Aloantibodies	Sensibilized	No therapy	Phototherapy, exsangvino	IUT, hidrops Hb<60r/n
D	132	64 (48%)	43 (32.5%)	25 (18.9%)
C	13	9 (69%)	4 (31%)	0
c	9	5 (55%)	3 (33%)	1 (11%)
E	10	9 (90%)	1 (10%)	0
Total	164	87 (53%)	51 (32%)	26 (15.8%)

There was a research of the reasons for appearance of alloimmunization at pregnant women. The most frequent reason is a lack of RhIG prophylaxis: post partum in 69.2% and during pregnancy in possible sensibilized conditions 28.6%. In 3.2% of the cases we acquired a data for post partum prophylaxis, in cases of not executed antenatal prophylaxis, and there is no data for a sensibilised attack.

Discussion

Severe HDFN is almost always caused by antigens with a protein biochemical structure. These antigens develop in the IVth month of pregnancy. Thus, for example, antigen D can be proved present on the erythrocyte membrane on the 38th day of gestation, which enables early primary and especially secondary immunization of the mother by creating anti RBC's antibodies of IgG class [2].

According literature data, the percentage of RhD alloimmunization during pregnancy has declined from 14% to 1% and 2% after introduction of postnatal prophylaxis with Rh immunoglobulin (RhIG) in 1960. This percentage globally further on declined to 0.1% with implementation of additional antenatal RhIG prophylaxis in 1979 [6, 7].

Still, a hemolytic disease of the fetus and the newborn caused by anti-D is one of the most often reason for HDFN in most underdeveloped countries in the world. The results acquired from most of the studies demonstrate that around 80% of HDFN belongs to RhD antibodies, or in combination with other antibodies of the Rh system 96.5% [7, 8]. In around 12% it is about antibodies towards other Rh antigens, mostly E and c [15]. The rest of the antibodies are towards Kell and Kid blood type

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systems [13, 14]. It is well known that the strength of HDFN varies according the characteristics of the antibodies, their amount and exposition on the fetus. Out of the outmost importance are the subclasses of IgG antibodies, that with the mother are most often of IgG2 class and do not cause lysis of Foethal RBC, which explains the fact that 53% of the D antigen allosensibilisation demonstrate no signs of HDFN, or it shows very weak symthoms. However, IgG1 and IgG3 subclasses, especially when present together, display a clinical picture of severe HDFN.

Allosensibilization to the RhD antigen during pregnancy requires invasive monitoring and treatment during the period of the whole pregnancy. Unfortunately, that does not exclude the possibility of unsuccessful pregnancy finish and the severity of the HDFN symptoms increases with every following pregnancy. Literature data show that by successful management, 86-90% of allosensibilized pregnancies reach to survival in the neonatal period [9, 10]. Data from CESDI for England and Ireland display that the fetal loss because of RhD sensibilization amounts 15-17% to 24 gestation week, and premature birth is about 35-48%.

There are two reasons why RhD immunization and sensibilization at pregnant women appear. Firstly, there is a lack of RhIG administration or inadequate prenatal protection, because all RhD negative women do not get anti-D immunoglobulin when necessary. Secondly, the reason is an occurrence of small, undetectable ("silent") fetomaternal bleedings in the third trimester of pregnancy [16, 17].

The quantification of the antibodies was executed by titer in all samples that have shown positive screening and identification to anti-RBC's antibodies. The Titer score elaborated by a microglutination test (MT) was at least two dissolution rates higher compared to the test-tube technique (TT-LISS).

The titer antibody is not a precise method, and can be useful only if executed in standard conditions and by an experienced staff. There are more standard methods for quantification of anti-RBC's alloantibodies: ELISA, flowcytometry, radioimmunoassay, that is not used as a routine [11]. In order to determine the border line titer in case of possible fetal suffer and correlation of alloantibodies titer with the severity of HDFN, additional research are needed which have not been included in this research study. So far, we should mention that the titer level of alloantibodies has a clinical significance only in the first pregnancy when the sensibility occurred. Following the titer rate in the next pregnancy is irrelevant, while a significant importance is shown by a sudden raise of titer for 2 or more dissolutions which implies significant changes and requires more detailed analysis [12].

The border line titer in case of possible fetal anemia, according scientific research is 1:34 (1:62) for TT-LISS and 1:16 for MT (because of the fact that the titer in MT are 2 to 3 times higher dissolutions) which is particularly subjective, since MT has relevant values and each increase requires additional explanation.

Conclusions

1.   The comparison of HDFN symptoms and therapy of newborn babies with immunohemolitic research has proven that:

- 53% of the antibodies did not provoke HDFN or it had mild symptoms and no need of therapy;

- 51% provoked moderate HDFN;

- 15.8% provoked HDFN with clinical signs of severe anemia, hidrops and fetus mortus.

2.   Out of total 216 alloantibodies, 81% (175) had clinical significance.

3.   The most frequent reason for severe hemolytic disease is the anti-D antibody in 61.1% of the cases. Out of them:

- 32.5% of the sensibilized to this antigen showed symptoms of moderate and severe rate of HDFN, with a need of phototherapy or/and exsangvino transfusion;

- 18.9%showed symptoms of severe fetal suffer, with a hydropic or dead fetus;

- Almost half (48%) of the D positive newborns of mothers with detected anti-D antibodies were without, or with a mild HDFN and had no need of therapy.

4.   In 15% of the cases it is about alloantibodies of other Rh antigens, mostly anti-C 7.8%, anti-E 6% and anti-c 5.4%.

5.   Anti-c is the only antibody that showed a potential for severe HDFN similar to the anti-D.

6.   Most frequent reason for alloimmunization of the mother is the lack of RhIG prophylaxis (97.8%): postnatal, antenatal and in case of possible sensibilized conditions during pregnancy.

7.   It is necessary to elaborate and adopt a national program for RhIG prophylaxis in Republic of Macedonia.

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