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# Introduction of maternal and foetal morphological parameters of blood for calculation the postpartum anti-RhD immunoglobulin doses**

# Wprowadzenie matczynych i płodowych parametrów morfologicznych krwi do obliczania dawek immunoglobuliny anty-RhD stosowanej po porodzie

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S u m m a r y

**Introduction.** Doses of anti-RhD Ig for immunoprophylaxis of haemolytic disease of newborn (HDN) are established with reference to the volume of RhD positive foetal red blood cells in the circulation of RhD negative person. However, results obtained from flow cytometry, microscopic Kleihauer-Betke test or serological tests represent percentage of foetal cells in mother's blood sample. Since 20 $\mu$g of anti-RhD can neutralise 1 ml of foetal RBCs, it is important to estimate the FMH volume in order to apply the appropriate dose of anti-RhD Ig.

**Methods.** Two formulas: 1st with average values of blood count and 2nd based on mother's body weight and Hct, maternal and foetal MCV measured in 58 women and their newborns.

**Results.** Mean values of measured maternal morphological parameters were close to the average values in population of women and newborns but the ranges of these used for FMH volume calculation were wide ex. Hct 25.7-46.2%, body weight 48-99 kg, calculated volume of maternal blood 3600-7425 ml and ratio of foetal MCV to maternal MCV 1.04-1.41. For 1% FMH we predicted 4 doses of anti-RhD Ig 750 IU (150 $\mu$g) using the 1st formula and using 2nd formula 2, 3, 4, 5 or 6 doses depending on the woman and her newborn parameters.

**Conclusions.** The formula with individual maternal and foetal morphological parameters is much more accurate for calculation of the volume of FMH and anti-RhD Ig doses compared with routinely used formula with average values.

Key words: feto-maternal haemorrhage (FMH), anti-RhD immunoglobulin, haemolytic disease of foetus/newborn (HDFN), immunoprophylaxis of HDFN

S t r e s z c z e n i e

**Wprowadzenie.** Dawki Ig anty-RhD stosowane w immunpoprofilaktyce choroby hemolitycznej noworodka ustalono w odniesieniu do objętości RhD dodatnich krwinek płodowych w krążeniu RhD ujemnych osób. Natomiast wyniki uzyskane z cytometru przepływowego, mikroskopowego testu Kleihauera-Betke lub testów serologicznych przedstawiają procentową zawartość krwinek płodowych w próbce krwi matki. Ważne jest obliczenie objętości krwinek płodowych, a następnie zalecenie odpowiedniej dawki IgG anti-RhD, gdyż 20 $\mu$g przeciwciał anti-RhD neutralizuje 1 ml płodowych krwinek czerwonych.

**Metody.** Dwa wzory do obliczania objętości krwinek płodowych: pierwszy ze średnimi wartościami parametrów krwi i drugi uwzględniający ciężar ciała matki, jej Ht, MCV oraz MCV krwinek płodowych zastosowano dla 58 kobiet i ich noworodków.

**Wyniki.** Średnie wartości mierzonych parametrów morfologicznych były zbliżone do średnich wartości w populacji kobiet i noworodków, ale zakresy wartości użytych do wyliczania objętości krwawienia płodowo – matczynego miały szeroki zakres i wynosiły: Ht 25,7-46,2%, ciężar ciała 48-99 kg, obliczona objętość krwi matki 3600-7425 ml i stosunek MCV noworodka do MCV matki 1.04-1.41. Dla przecieku krwinek płodowych stanowiącego 1% przewidywano 4 dawki Ig anty-RhD 750 IU (150 $\mu$g) stosując pierwszy wzór i 2, 3, 4, 5 lub 6 dawek zależnie od kobiety i jej noworodka oraz zastosowania drugiego wzoru.

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**Wnioski.** Wzór zawierający indywidualne matczyne i płodowe parametry morfologiczne pozwala obliczyć objętość krwinek płodu, a następnie ustalić odpowiednią dawkę Ig anty-RhD dużo dokładniej niż wzór z wartościami przeciętnymi, który zazwyczaj jest stosowany.

Słowa kluczowe: krwawienie płodowo-matczyne, immunoglobulina anty-RhD, choroba hemolityczna płodu/noworodka (ChHPN), immunoprofilaktyka ChHPN

## INTRODUCTION

Detection and quantification of foetal red blood cells in maternal blood samples is essential for obstetrical management in cases of pathological massive feto-maternal haemorrhage (FMH) and also determination of an accurate standard dose of anti-RhD immunoglobulin (Ig) in antepartum and postpartum immunoprophylaxis of haemolytic disease of foetus/newborn (HDFN). As some investigators we also have introduced various methods of detection and quantification of foetal red blood cells such as flow cytometry tests, the Kleihauer-Betke microscopic test and the serological test. All obtained results are presented as a percentage of foetal red blood cells among mother red blood cells. However, doses of anti-RhD Ig are established in relation to the volume of RhD positive foetal red blood cells in the circulation of RhD negative persons.

## AIM OF THE STUDY

The aim of this study was to calculate volume of FMH and then estimated doses of anti-RhD Ig using two different formulas based on average or individual morphological parameters of tested mothers and their newborns.

## MATERIALS

EDTA blood samples from 58 mothers obtained in 2 hours after delivery and 58 cord blood samples from their newborns.

## METHODS

The blood count was measured by the haematological analyser (Beckman Coulter, USA).

The clinical history of childbirths and information about body weight of mothers were obtained from the clinic.

Two formulas were used for the calculation of FMH volume (ml of foetal red blood cells):

1. $$\frac{\text{Percentage of foetal cells x 1800 ml x 1.22}}{100}$$

where 1800 ml is an average volume of red blood cells in women's circulation and 1.22 is an average proportion of the newborn's MCV (mean corpuscular volume) to the mother's MCV;

2. $$\frac{\text{Percentage of foetal cells x 75 ml x mother's body weight x her Hct x newborn's MCV: mother's MCV}}{100}$$

where the volume of red blood cells in each mother was estimated from her body weight and Hct and based on presumption that there is 75 ml of blood for each 1 kg of body weight, and MCV is tested in each mother and newborn.

Formulas above were used for hypothetic FMH 1%, which could happen in each of tested women.

## RESULTS

The age of 58 investigated women was ranging from 18 to 42. Thirty three of them were primigravida and 25 had one or more deliveries in the past. Thirty four had a natural delivery, and 24 women were delivered by caesarean section, all at a normal term. None of the women had excessive blood loss, which required transfusion of red blood cells. FMH measured by various methods in 55 maternal samples was $\leq$ 0.1%, in some remaining samples: 0.15%, 1.45%, 3.5%.

The following parameters were used to calculate the volume of FMH: the newborn's MCV and the mother's MCV to calculate their ratio, the Hct of mother to calculate the volume of her red blood cells in her circulation, the body weight of mother to estimate the volume of her blood.

Table 1 presents ranges of these parameters, mean values and standard deviations. Mean values of our patients' parameters were similar to the average values used in the 1st formula. However, the individual parameters revealed wide ranges. The FMH volume estimated with 1st formula was different in comparison to the FMH volume for the same percentage but calculated with 2nd formula. Table 2 shows estimated volumes of foetal red blood cells and the number of predicted standard doses of anti-RhD Ig, if their contents were 1% in all tested samples.

## DISCUSSION

HDNF prophylaxis by giving anti-RhD Ig to RhD negative women after delivery RhD positive newborn was introduced in late sixties. It is assumed that 25 $\mu$g of RhD Ig is enough to neutralize 1 ml of RhD positive red blood cells of newborn (1-4) but some authors deem that already 20 $\mu$g is sufficient for this purpose (5-10). There is no clear evidence which dose of anti-RhD IgG

Table 1. Morphological parameters of 58 mothers and their newborns used for calculation the FMH.

| Newborn's MCV [fl] | | Mother's MCV [fl] | | Mother's Hct [%] | | Mother's body weight [kg] | | Calculated volume of mother's blood cells [ml] | | Newborn's MCV / Mother's MCV | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| range | x ± SD | range | x ± SD | range | x ± SD | range | x ± SD | range | x ± SD | range | x ± SD |
| 100.3-119.2 | 109.8 ± 4.5 | 70.9-102.2 | 91.8 ± 5.7 | 25.7-46.2 | 34.2 ± 4 | 48-99 | 70 ± 12 | 1177-2943 | 1797 ± 376 | 1.04-1.41 | 1.20 ± 0.08 |

Table 2. Predicted numbers of anti-RhD IgG doses administered postpartum, based on the FMH volume 1% and calculated by two formulas.

| Formulas | FMH | | The number of doses | |
|---|---|---|---|---|
| | % | volume (ml) | 100* μg (5 ml)[1] | 150** μg (7.5 ml)[1] |
| 1 | 1 | 22 | 5 | 4 |
| 2 | 1 | 13-38 | 3-8 | 2-6 |

*standard dose used in UK, **standard dose used in our country
[1]in parentheses: volume of neutralized RBCs

is the most effective and safe. Various standard doses after physiological delivery are used in various countries: 500, 600, 750, 1000, 1250 or 1500 IU (100, 120, 150, 200, 250 or 300 μg).

Many laboratories in the world evaluate amount of FMH using a 50-year-old Kleihauer-Betke test, agglutination technique (rosette test, DiaMed gel) an modern flow cytometry assays. Results obtained are presented as a percentage of foetal red blood cells among maternal red blood cells (2,11-16). To determine accurate dose of anti-RhD Ig usually Mollison formula is used (1, 3, 4, 6, 11, 12, 14, 17, 18). It assumes that the maternal red blood cell volume is 1800 ml and foetal cells are 22% larger than maternal cells (foetal MCV: maternal MCV = 1.22). Some authors postulate to take into consideration body weight and height while establishing mother's volume of blood (19-21). Investigators from Collage of American Pathologists (CAP) found that 20% of 1450 laboratories recommended incorrect doses of anti-RhD Ig for the volume of foetal red blood cells they had measured (21). Most of the laboratories in cited study used Mollison formula with average values. CAP suggests to use "RhIg Dose Calculator", which takes into account the mother's actual blood volume based on her weight and height thereby limiting mathematical errors.

Our investigation shows that average body weight, haematocrit and estimated blood volume and red cells volume of women after delivery do not differ significantly from those which are regarded as average, however, the range of each parameter is wide.

Considering the same hypothetic percentage of newborn's red blood cells in mother's blood and applying formula which require more individual data of mother and her baby we obtained significant differences between calculated blood/red blood cells volumes and doses of anti-RhD Ig for the same woman.

## CONCLUSIONS

1. Formula for FMH volume calculation from its percentage which takes into account individual parameters, like body weight, Hct, MCV of mother and newborn, allows to determine more accurate dose of anti-RhD Ig for each woman than formula with the average values for population.
2. Determination of some morphological parameters of mother's and newborn's blood and mother's body weight is contained in routine care of mother and child on obstetric ward and is easy to obtain.

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