LITHUANIAN UNIVERSITY OF HEALTH SCIENCES
Faculty of Medicine
Department of Obstetrics and Gynaecology

Title of Master’s Thesis:
FETAL ANAEMIA IN RH ISOIMMUNISED PREGNANCIES

A Dissertation submitted in Partial Fulfilment of the Requirements
for the Degree Master of Medicine
Lithuanian University of Health Sciences

Author:
Emma Maria Lindberg

Supervisor:
assoc. prof. Regina Mačiulevičienė

Kaunas
2019
# TABLE OF CONTENTS

SUMMARY .................................................................................................................. 3

ACKNOWLEDGEMENTS ............................................................................................ 5

CONFLICTS OF INTEREST ......................................................................................... 6

ETHICS COMMITTEE CLEARANCE ........................................................................... 7

ABBREVIATIONS ........................................................................................................ 9

CHAPTER 1: INTRODUCTION ...................................................................................... 10

CHAPTER 2: AIM AND OBJECTIVES .......................................................................... 11

CHAPTER 3: LITERATURE REVIEW ............................................................................. 12

  3.1 EPIDEMIOLOGY .................................................................................................... 12

  3.2 PATHOGENESIS .................................................................................................... 12

  3.3 MANIFESTATIONS ................................................................................................ 13

  3.4 DIAGNOSIS .......................................................................................................... 14

    3.4.1 Diagnosis – Detection of women at risk of Rh sensitization ......................... 14

      3.4.1.1 ABO, Rh-D determination ........................................................................ 14

      3.4.1.2 Non-invasive prenatal testing ................................................................. 14

      3.4.1.3 Rosette screen ........................................................................................ 15

      3.4.1.4 Kleihauer – Betke test .......................................................................... 15

      3.4.1.5 Indirect antiglobulin test ...................................................................... 15

    3.4.2 Diagnosis – Detection of fetal anemia ......................................................... 16

      3.4.2.1 Doppler ultrasound ............................................................................. 16

      3.4.2.2 Invasive diagnostics ......................................................................... 17

  3.5 PROPHYLAXIS ...................................................................................................... 18

  3.6 TREATMENT/MANAGEMENT ............................................................................ 19

  3.7 OUTCOMES ......................................................................................................... 22

CHAPTER 4: METHODOLOGY ..................................................................................... 23

CHAPTER 5: RESULTS .................................................................................................. 24

CHAPTER 6: DISCUSSION ........................................................................................... 28

CHAPTER 7: CONCLUSION ......................................................................................... 31

CHAPTER 8: PRACTICAL RECOMMENDATIONS ...................................................... 32

REFERENCES ........................................................................................................... 33
SUMMARY

**Author name:** Emma Maria Lindberg

**Research title:** Fetal anemia in Rhesus (Rh) isoimmunised pregnancies

**Aim:** To assess the value of Doppler ultrasound peak velocity in middle cerebral artery (MCA) and splenic artery in cases of Rhesus isoimmunisation to predict fetal anemia.

**Objectives:**

1. To determine the value of the peak systolic velocity in MCA for the prediction of fetal anemia in Rh isoimmunised pregnancies.
2. To determine the value of Doppler velocimetry of the splenic artery for prediction of fetal anemia in Rh isoimmunised pregnancies.
3. To compare the value of the Doppler ultrasound peak velocity of the MCA and the splenic artery for the prediction of fetal anemia in Rh isoimmunised pregnancies.
4. To determine the correlation between the change in middle cerebral artery peak systolic velocity (MCA-PSV) and splenic artery (SpA-PSV) with the change in fetal hemoglobin (Hb) immediately following intrauterine transfusion (IUT).
5. To determine the correlation between the change in MCA-PSV and SpA-PSV with the change in fetal hematocrit (Hct) immediately following IUT.

**Methodology:** This retrospective observational study was conducted at the Hospital of Lithuanian University of Health Sciences Kaunas Clinics, department of Obstetrics and Gynaecology. In our study we included all previous cases with IUT due to Rh isoimmunisation. A total number of 20 cases with IUT were included from January 1, 2004, through March 31, 2019.

**Results:** A total of twenty intrauterine blood transfusions to ten pregnancies with Rh isoimmunisation were included to this study. The mean pre-transfusion MCA-PSV was 1,84±0,46 multiples of mean (MoM) and the SpA-PSV was 1,91±0,55 MoM. The post-transfusion MCA-PSV was 1,13±0,22 MoM and the SpA-PSV was 1,42±0,23 MoM. Measurements that were taken 24 hours after transfusion; the mean MCA-PSV was 1,45±0,21 MoM. Statistically significance between pre- and post-transfusion values was present in MCA-PSV MoM and SpA-PSV MoM. Pre-transfusion correlation between MCA-PSV MoM and Hb was weak (r=-0,273), between SpA-PSV MoM and Hb (r=-0,850) and between SpA-PSV MoM and Hct (r=-0,833) was very strong. Post-transfusion correlation between MCA-PSV MoM, Hb and Hct was strong (r=-0,609 and r=-0,649). The correlation between post-transfusion SpA-PSV MoM, Hb and Hct was weak (r=-0,217 and r=-0,233). The mean pre-transfusion
Hb and Hct were 50.47± 20.75 g/l and 15.47±6.05%, respectively. Post-transfusion mean of Hb and Hct was 113.60±29.32 g/l and 34.33±8.93%, respectively.

**Conclusions:** The peak systolic velocity of middle cerebral artery and splenic artery measured by Doppler velocimetry is efficient methods for the prediction of fetal anemia in Rh isoimmunised pregnancies. These two methods for prediction of fetal anemia are relatively different, where in this study; the SpA-PSV MoM is the better predictor. Strong correlations between post-transfusion MCA-PSV MoM and fetal Hb & Hct are observed. However, due to the lack of data in some cases, correlation between post-transfusion SpA-PSV MoM and fetal Hb & Hct are inconclusive.

**Recommendations:** We suggest including Doppler evaluation of both MCA and splenic artery into the management guidelines of rhesus isoimmunised pregnancies. Examination with Doppler velocimetry should be conducted to evaluate the presence and severity of fetal anemia. Especially focus on the velocimetry of splenic artery seeing that according to this study, it is a better predictor of fetal anemia in case of Rh isoimmunisation. In order to obtain more accurate results in the future, the Doppler evaluation of both arteries should be performed pre-IUT, post-IUT and 24 hours after IUT.

**Key words:** Rh isoimmunisation, fetal anemia, middle cerebral artery, splenic artery, peak systolic velocimetry, and intrauterine transfusion.
ACKNOWLEDGEMENTS

I would like to thank my supervisor Associate Professor Regina Mačiulevičienė for her guidance and encouragement throughout my research. She has been a true inspiration source.

Moreover, I also would like to thank Associate Professor Anna Casselbrant from Academy of Salgrenska, University of Gothenburg, who has been a support and helped me when questions related to SPSS has emerged.

Finally I would like to thank specialty trainee Marija Paulionytė for all the time she invested in order to help me create this thesis, it wouldn’t have been possible to finish it without her guidance and support.
CONFLICTS OF INTEREST

The author reports no conflicts of interest.
ETHICS COMMITTEE CLEARANCE

The agreement of the independent Local Ethical Committee of Kaunas University of Medicine was obtained prior to the study.

Title:
Fetal anemia in Rhesus (Rh) isoimmunised pregnancies

Number:
The committee clearance number is BEC–MF–39.

Date of issue:
2018-11-12
DĖL PRITARIMO TYRIMUI

LSMU Bioetikos centras, įvertinė Emma Maria Lindberg pateiktus dokumentus, studentės tiriamajam darbui tema „Fetal anemia in Rh isoimmunized pregnancies“ pritaria*.

* Pastaba: Šis pritarimas neatliežia tiriamajį mokslinį darbą vykdančių asmenų nuo prievolės laikytis Bendrojo duomenų apsaugos reglamento nuostatų ir nuo atsakomybės gauti nacionalinio arba regioninio bioetikos komiteto leidimą, jei toks leidimas būtinas pagal LR Biomedicinių tyrimų etikos įstatyme numatytais reikalavimus.
ABBREVIATIONS

cffDNA – Cell-free fetal DNA
DAT – Direct antibody titer
FMH – Fetomaternal hemorrhage
Hb – Hemoglobin
HbF – Fetal hemoglobin
Hct – Hematocrit
HDFN – Hemolytic disease of the fetus and newborn
IAT – Indirect antiglobulin test
IgG – Immunoglobulin G
IgM – Immunoglobulin M
IUT – Intrauterine transfusion
IVIG – Intravenous immunoglobulin
LOTUS study – LOng-Term follow- up after intra-Uterine transfusionS
MCA – Middle cerebral artery
MCA-PSV – Middle cerebral artery peak systolic velocity
MoM – Multiples of medians
NIPT – Non-invasive prenatal testing
P – probability value
RBC – Red blood cells
RES – Reticuloendothelial system
Rh – Rhesus
RhIg – Rh-D immunoglobulin
SD – Standard deviation
SpA-PSV – Splenic artery peak systolic velocity
SPSS – Statistical Package for Social Science
TPE – Therapeutic plasma exchange
CHAPTER 1: INTRODUCTION

Anemia continues to be an infrequent but life-threatening condition for the developing fetus. Rhesus isoimmunisation is a frequent cause of fetal anemia, caused by transportation of maternal antibodies from the placenta and subsequently causing hemolysis of fetal blood cells.

Before 2000, the detection methods of fetal anemia were either invasive (such as cordocentesis, amniocentesis) or undependable or late (ultrasound signs of hydrops). Year 2000, Mari et al. confirmed by their study that moderate to severe anemia can be established noninvasively by measuring MCA-PSV to more than 1.5 MoM, in fetuses at risk of maternal RBC isoimmunisation [1,2]. When severe fetal anemia is suspected by Doppler ultrasound, cordocentesis is necessary for assessment of fetal Hb concentration and thereby to determine if an indication of IUT is present. The anemia was classified into: severe fetal anemia – Hb <90 g/L, moderate fetal anemia – Hb 90-120 g/L, mild fetal anemia – Hb 120-145 g/L and normal values were considered when Hb > 145 g/L.

The prophylaxis for prevention of rhesus isoimmunisation, anti-D immunoglobulin (Ig), was developed over 50 years ago and has ever since been the gold standard in the prevention approach for the anti-D sensitized pregnancies[3]. Although adequate antenatal and postnatal anti-D Ig prophylaxis for Rh isoimmunisation are gold standard, fetal hemolytic disease still continues to occur in Lithuania. The overall worldwide frequency of anti-D sensitized pregnancies is less than 3%, which makes it uncommon but still significant[4]. Because of the infrequent occurrence, not a lot of studies are being conducted in the topic. The literature is limited to case reports, hence there is a restricted access of information regarding diagnostic opportunities (most optimal approach) and effectiveness of treatment.

In the current study, we aim to assess the value of Doppler ultrasound MCA-PSV and SpA-PSV in cases of Rh isoimmunisation to predict fetal anemia. Another aim is to determine the correlation between the change in MCA-PSV and SpA-PSV with the change in fetal Hb and Hct immediately following IUT. And additionally to assess which diagnostic method for the prediction of fetal anemia, MCA-PSV or SpA-PSV, is most accurate.

This study could help to improve the diagnostic approach for those anti-D sensitized women and would probably improve the outcomes of newborns suffered from fetal anemia. Due to this, we decided to conduct a retrospective observational study of all cases where intrauterine blood transfusion was performed, in order to obtain optimal diagnostic approach in cases of Rh isoimmunisation.
CHAPTER 2: AIM AND OBJECTIVES

**Aim:** To assess the value of Doppler ultrasound peak velocity in MCA and splenic artery in cases of Rhesus isoimmunisation to predict fetal anemia.

**Objectives:**

1. To determine the value of the peak systolic velocity in MCA for the prediction of fetal anemia in Rh isoimmunised pregnancies.
2. To determine the value of Doppler velocimetry of the splenic artery for prediction of fetal anemia in Rh isoimmunised pregnancies.
3. To compare the value of the Doppler ultrasound peak velocity of the MCA and the splenic artery for the prediction of fetal anemia in Rh isoimmunised pregnancies.
4. To determine the correlation between the change in MCA-PSV and SpA-PSV with the change in fetal Hb immediately following IUT.
5. To determine the correlation between the change in MCA-PSV and SpA-PSV with the change in fetal Hct immediately following IUT.
CHAPTER 3: LITERATURE REVIEW

3.1 EPIDEMIOLOGY

Since the implementation of Rh-D immunoprophylaxis in 1968, there has been a drastic decrease in the incidence of Rh isoimmunisation[5]. The occurrence of fetal anemia due to Rh isoimmunisation is highly dependent on the socio-economical status of the country. Higher incidence occurs in developing countries, where the possibility of diagnosis and prophylaxis may be limited. The worldwide frequency of incidence ranges is less than 3%. Nevertheless every year in the United Kingdom, there are around 600–700 new cases of Rh isoimmunisation. In Lithuania per year, there are approximately 50 cases of Rh isoimmunisation. The suggested reasons for isoimmunisation are either due to insufficient prophylactic dose or failure to cover a potentially sensitizing event[6].

3.2 PATHOGENESIS

There has been many theories regarding the pathogenesis of fetal anemia over the years, but the latest research by Abbasi et al. may provide us with the newest information[7]. Rh isoimmunisation is the development of antibodies against the Rh antigens present on the surface of red blood cells (RBC). It occurs when Rh-positive RBCs from the fetus enters the maternal circulation, which is Rh negative, usually through fetomaternal hemorrhage (FMH)[7]. Scientific researches has proven that FMH occurs throughout the whole pregnancy, it is only the amount of hemorrhage that differs between the trimesters[6]. A Kleihauer–Betke Test is a standard method to determine this amount (the test will be further discussed under “3.3 Diagnosis”). When fetal Rh-positive RBCs enters the maternal circulation, the Reticuloendothelial system (RES) recognizes the fetal RBC as foreign antigen and responds with the formation of IgM antibodies against Rh-antigens. During the first exposure of fetal RBC, the maternal immune system only produces a small amount of IgM (initial response). These IgM antibodies cannot cross the placenta and therefore does not affect the pregnancy. When a second exposure occurs, usually during a second pregnancy with Rh-positive fetus, memory B-cells responds stronger by producing IgG antibodies. These IgG antibodies cross the placenta and binds to Fc-gamma receptors present on the syncytiotrophoblasts[6]. The antibody-coated RBC are hemolyzed in the fetal spleen, which causes anemia, hydrops and finally fetal death[7]. The pathogenesis is also illustrated figuratively in Fig. 1.

The most frequent antigen that causes severe Rh isoimmunisation is Rhesus D antigen, which is also the most important Rh[6]. Other Rh antigens such as c, E and Kell antigens, these antigens have
a potential to cause severe isoimmunisation reaction but they are less frequent. Rh antigens (such as Duffy, Kidd, M and S), they rarely cause any significant problems.

3.3 MANIFESTATIONS

The manifestations of fetal anemia usually manifest clinically as decreased fetal movements. The progression of the condition evolves into extramedullary erythropoiesis in the spleen and liver. This is seen during an ultrasound examination as hepatosplenomegaly. In severely anemic fetus increased cardiac output is seen, which will develop into heart failure due to the cardiac hypoxia. Heart failure manifestations are demonstrated sonographically as hydropic changes, such as pericardial effusion, subcutaneous edema, scalp edema and pleural effusion. Due to the compensation of reduced oxygen supply, the manifestation of placentomegaly can be seen ultrasonographically[5,6].
3.4 DIAGNOSIS

3.4.1 Diagnosis – Detection of women at risk of Rh sensitization

3.4.1.1 ABO, Rh-D determination

During the initial antenatal visit, it is strongly recommended for all pregnant women to be screened for Rh isoimmunisation including blood type (ABO, RhD) and antibody detection test (see “3.3.1.5 Indirect antiglobulin test”) [5]. In case of a Rh-D positive women, there is no indication to proceed further tests. On the other hand, if the expectant mother is Rh-D negative paternal testing is recommended. If the father is tested Rh-D positive, it is recommended to perform genotyping of the paternal Rh-D coding gene. In case of homozygosity, the offspring will inherit the Rh-D gene and there are potential for sensitization. There is a 50% probability for the fetus to be Rh-D positive, if the father has a heterozygous gene [6]. The red cell genotyping of the fetus can be possible by obtaining fetal DNA from maternal serum (see “3.3.1.2 Non-invasive prenatal testing”) [8].

3.4.1.2 Non-invasive prenatal testing

The red cell genotyping of the fetus by non-invasive prenatal testing (NIPT), is recommended when the paternal Rh-D coding gene is heterozygous [8]. Until recently, evaluation of Rh-D status in fetus has only been available by amniocentesis [9]. NIPT evaluates the cell-free fetal DNA (cffDNA), which is believed to be derived from trophoblasts. CffDNA are very pregnant-specific, since they are rapidly removed from maternal circulation after delivery. Lo et al. made the first detection of cffDNA in 1997 [10]. NIPT is now available in many different countries around the world, including Lithuania since year 2016, for the purpose to detect fetal Rh-D genotype but also to provide guidance in the use of antenatal prophylaxis by anti-D immunoglobulin [11]. This approach of NIPT is currently being offered in Denmark and the Netherlands between 25–28 weeks’ of gestation, but research has established that maximized benefits can be accomplished by an earlier Rh-D genotyping. This method would provide a reduction of costs and risks of unnecessary anti-D administration [12]. In Sweden and in the UK, pilot programs where fetal Rh-D genotyping were implemented in the first trimester and at 16 weeks’ gestation has demonstrated comparable results [13]. Implementation of cffDNA screening for the status of fetal Rh-D would prevent up to 40% of the application of Rh-IG to Rh-D negative women, which is an ethical and practical approach given the world-wide shortage of this product [14].
3.4.1.3 Rosette screen

A highly sensitive screening method to detect fetal D+ red cells in maternal Rh-negative blood. The rosette test is inexpensive, easily conducted and can detect 10 mL or more of the fetal blood in the maternal circulation. A positive result, requires quantification of the FMH by Kleihauer-Betke acid elution test, in order to determine the correct dose of RhIg required for prophylaxis[15].

3.4.1.4 Kleihauer – Betke test

The purpose of this test is to quantify the amount of fetal blood transferred to the maternal circulation. In 1957, the Kleihauer–Betke test was initially described by Kleihauer, Braun and Betke, which rely on the principle that fetal RBCs mostly contain fetal hemoglobin (HbF)[15]. These HbF has a different resistance to acid, when compared with adult Hb. Which makes it possible to differentiate maternal Hb from HbF, by adding acid elution to a smear with maternal blood on[6]. This method is not without limitations, including a low sensitivity, poor reproducibility and an inclination to overestimate the quantity of the hemorrhage[15].

3.4.1.5 Indirect antiglobulin test

The ability to detect sensitization of mother is based on presence of anti-D antibodies in the maternal circulation[5]. The indirect antiglobulin test (IAT) gives us the possibility to incubate maternal RBC (carrying Rh-antigen), and to analyze the agglutination reaction, which would occur after adding anti-human immunoglobulin. The titer is determined by the maximum dilution that produces an agglutination reaction[6]. The estimation of maternal sensitization are beneficial in two main ways: A low titer of antibodies suggests that the infant might be unaffected or slightly affected, while an increased titer insinuates a warning of a increased hemolytic process[16]. The level of antibody titer that is referred as a critical value ranges from 8 to 32 in the scientific literatures[4]. Any antibody identified is quantified and when exceeding 1/16, monitoring by Doppler measurement of MCA-PSV and SpA-PSV is indicated. Research has been able to show a direct correlation between the levels of antibody titer with the maternal parity. Therefore more the parity, higher the IAT would be. Philip el al. established as well a connection where severity and fetal outcome are in direct relation with the antenatal maternal serum IAT titer[16].
3.4.2 Diagnosis – Detection of fetal anemia

3.4.2.1 Doppler ultrasound

Predicament of fetal anemia by using Doppler ultrasound examination of MCA-PSV is an important method in the monitoring of fetal wellbeing. It has become a goal standard of method in diagnosing of fetal anemia noninvasively[17]. The Doppler ultrasound enables identification of anemic fetuses by displaying an accelerated blood velocity in the MCA[1,2]. The accelerated blood velocity is believed to be due to decreased blood viscosity and shunting of the blood away from the periphery[18].

Mari et al. describes the most commonly used Doppler ultrasound technique for the examination of MCA-PSV. The technique describes how measurements of the MCA are obtained with an axial section, which includes the thalami and the cavitas septi pellucidi. By color Doppler, the circle of Willis is visualized, and the MCA is examined shortly after its origin from the internal carotid artery. Preferably, the angle between the ultrasound beam and the direction of MCA blood flow should be as close to zero as possible and parallel to the artery for the entire length, so no need for angle correction. It is recommended to obtain measurements at the highest point of at least three consecutive waveforms[19].

Preceding studies have established the correlation between level of fetal Hb and the MCA-PSV. In cases of mild anemia, minimal or no augmentation of blood velocity can be seen, hence the difficulties in diagnosing fetal anemia early. An escalation of MCA blood velocity is seen in cases of moderate and severe fetal anemia, therefore the detection accuracy of anemia improves[20]. According to Zimmermann et al. a positive correlation of 98% between fetal anemia and increased values of MCA-PSV has been seen[21]. Normograms of MCA-PSV are available and the different values are assembled according to MoM. In case of MCA-PSV has exceeded levels of 1.5 MoM; the association with significant fetal anemia is very considerable[6]. Measurements greater than 1.5 MoM are used as a screening test to identify the fetus suffering from severe anemia. Mari et al. reported in one of the first large multicentre studies, a sensitivity for moderate or severe anemia using a single value MCA-PSV of nearly 100% with a false-positive rate of 12%[1]. However, Pretlove et al. published a meta-analysis in 2009, where the diagnostic values of MCA Doppler flow studies for fetal anemia were also discussed. Twenty-five studies with over 1600 participants were included, where dated could be pooled from 9 of these studies. The meta-analysis reported a sensitivity of over 75% and a specificity of over 90% for severe anemia[22].

Several studies have also proven the usefulness of using MCA-PSV in the prediction of timing IUTs[8,23]. Detti et al. studied the MCA-PSV values in 64 fetuses before cordocentesis, which had already received an intrauterine blood transfusion. The MCA-PSV for the estimation of mild,
moderate and severe anemia at a sensitivity of 100% presented false-positive rates of 70%, 37%, and 6%, respectively[24]. However, after two IUTs the adult RBCs totally replace the fetal cells, and therefore the sensitivity of MCA-PSV for detection of fetal anemia decreases. Hence, adult RBCs have different physical characteristics than fetal RBCs. Adult RBCs are smaller, less rigid and have less oxygen-binding capacity, whereas fetal RBCs carry more oxygen but release less to tissue[25]. Cerebral Doppler thus remains a useful tool for monitoring fetuses at risk of anemia undergoing serial IUTs because of maternal isoimmunisation[24].

As it is well known, the spleen plays an essential role in red cell isoimmunisation. The spleen is both responsible for the destruction of the corpuscles, which were damaged by antibodies, and contributes to erythropoiesis as compensation for the severe anemia[26]. Only a few studies have been presented regarding the clinical value of SpA-PSV in cases of Rh isoimmunisation.

Abuhamad et al. and Bahado-Singh et al. describe the most commonly used Doppler ultrasound technique for the examination of SpA-PSV. The technique describes how main splenic artery is observed by color flow Doppler in an axial view of the fetal abdomen, where the splenic artery exits the celiac axis and enters the splenic hilum. Measurements are obtained from a straight segment of the vessel, and the angle of insonation should be close to zero degrees. It is recommended to obtain measurements at the highest point of at least three consecutive waveforms. The deceleration angle of the splenic artery is also measured, as it reflects the average rate of deceleration during diastole[27,28].

Bahado-Singh et al. provides convincing evidence of hyperdynamic circulatory changes by Doppler SpA-PSV as a result from the anemia. Correlation between increasing SpA-PSV and deficiency of Hb was significant. Hence, correct diagnosis by means of the deceleration angle, gives a false-positive rate of approximately 8%. Which would provide a reduction in rate of cordocentesis by >91%[27]. Bahado-Singh et al. made an interesting observation, which were that the SpA-PSV seemed to be more beneficial in predicting anemia after 27 weeks’ of gestation. After this gestational age, the spleen matures as an organ of erythropoiesis, which could be a probable explanation for this[26].

**3.4.2.2 Invasive diagnostics**

One of the invasive diagnostic tests that were previously used to assess the severity of fetal anemia was the quantification of bilirubin levels (OD450) in amniotic fluid upon amniocentesis. Each invasive diagnostic test carries an increased risk of complication, including spontaneous miscarriage and amniotic fluid leakage[29]. Oepkes et al. concluded in a prospective multicenter study, that the MCA-PSV could safely replace the invasive diagnostic testing in the managing of Rh-isoimmunised
pregnancies[24]. The fetal blood sampling by cordocentesis is mainly performed when the ultrasonography has shown a MCA-PSV >1.5 MoM for gestational age and increasing, hence indicates a moderate to severe fetal anemia. Since this procedure presents a 1-2% risk of fetal loss, with the highest possible risk at lower gestational age and in fetuses with hydrops. The fetal blood is collected for evaluation of Hct, Hb, blood group (ABO and Rh) and direct antibody titer (DAT) status[16]. In the case of non-acceptable fetal gestational age for delivery, and the level of Hct is <30% or Hb is more than one to two standard deviations below the mean value for gestational age, further treatment such as IUT are usually indicated[5].

3.5 PROPHYLAXIS

In the 1960s, the routine postnatal administration of anti-D intravenous immunoglobulin (IVIG) was introduced. Which has considerably decreased the maternal isoimmunisation identified in subsequent pregnancy from 15% to <2%. In the latter years, antenatal (administrated between week 28-34 of gestation) and postnatal prophylaxis with anti-D IVIG has become a standard approach in many developed countries[29]. Different possible sensitizing events that can occur during a pregnancy and may require anti-D IVIG are displayed in Table 1.

Koelewijn et al. reported in a nationwide observation study, that the administration of antenatal anti-D Ig reduces the risk of anti-D immunization by half when detected early in the next pregnancy[30]. According to Crowther et al. antenatal anti-D Ig did not significantly reduce the risk of immunization during the current pregnancy and until 12 months after birth. However, a significantly reduced possibility of having a positive Kleihauer test during the pregnancy and 12 hours after birth were reported. These results were achieved in two randomized controlled trials presented in a systemic review[31].

In at least two large-scale studies made in Denmark and in the Netherlands, have reported that by using non-invasive fetal RHD typing, antenatal anti-D administration can be restricted to only pregnant women carrying a Rh D positive child[32,33]. According to Clausen et al. the false-negative test results, by using fetal RHD typing in week 26 of gestation, is very low[32].
Table 1. Possible sensitizing events in pregnancy

<table>
<thead>
<tr>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antepartum hemorrhage/Uterine bleeding</td>
</tr>
<tr>
<td>Ectopic pregnancy</td>
</tr>
<tr>
<td>Evacuation of molar pregnancy</td>
</tr>
<tr>
<td>External cephalic version</td>
</tr>
<tr>
<td>Amniocentesis, chorionic villus biopsy and cordocentesis</td>
</tr>
<tr>
<td>Abdominal trauma</td>
</tr>
<tr>
<td>Intrauterine death and stillbirth</td>
</tr>
<tr>
<td>Miscarriage</td>
</tr>
<tr>
<td>In-utero therapeutic intervention (such as transfusions and surgery)</td>
</tr>
<tr>
<td>Therapeutic termination of pregnancy</td>
</tr>
<tr>
<td>Delivery – Normal, instrumental and Caesarean section.</td>
</tr>
</tbody>
</table>

3.6 TREATMENT/MANAGEMENT

The management of fetal anemia is a challenging task, since most of the affected fetuses are not matured enough to be delivered. Management of pregnancies at risk of rhesus isoimmunisation is described in Fig. 2.

The most current successful method in managing anemic fetuses in isoimmunised pregnancy is by IUT, but this procedure is not without its risks[6]. The volume transfused during an IUT is based on fetal gestation, fetal weight, fetal condition (e.g. presence of hydrops), Hct of the donor and the target final fetal Hct. The general assumption is that the target final Hct should be approximately 40-50%[8].

According to Weisz et al. and other studies, the mean survival rate both for hydropic and non-hydropic fetuses, after IUT is around 90%. Where a lower survival rate of the hydropic fetuses can be observed[29,34]. Whereas another study made by Osanan et al. reported a perinatal mortality rate associated with IUT of almost 20% in fetuses between weeks 19 to 34 of gestation[35]. Hence, the importance and desire to develop a non-invasive approach to manage anemic fetuses since IUT is associated with morbidity and mortality. Approaches combining therapeutic plasma exchange (TPE) and IVIG have presented potential in preventing fetal anemia in early gestation. It is still not clear by which mechanism the combination of TPE and IVIG therapy works to diminish the effects of maternal RBC isoimmunisation[23]. According to Lindenburg et al. the incidence of perinatal loss after an IUT procedure before 20 weeks of gestation is higher, than compared with the anemic fetuses that
underwent IUT after 20 weeks of gestation[36]. This is where the combination of TPE and IVIG can serve as a bridge by postponing the onset of hemolytic disease of the fetus and newborn (HDFN), delaying the requirement for IUT until appropriate gestational age is achieved[23].

There are studies where performing IUTs hasn’t been necessary, due to successful results from the combination of TPE and IVIG[37–39]. According to Bellone et al. their study with the protocol of four-TPE with a 3-week regimen, starting early in gestation with IVIG-dose given after the third TPE and continued weekly until 28 weeks of gestation, together with measurements of MCA-PSV throughout the gestation is effective in avoiding severe anemia until delivery without the aid of IUT[40]. However, a monotherapy with TPE has not been favorable, since TPE therapy has not been successful at maintaining the IgG depletion[41]. The reason for this, is believed to be due to the rate of IgG catabolism is slower at lower concentrations[40].

The optimal timing of delivery is difficult to decide, most expert opinions suggest to plan the delivery at 37-38 weeks of gestation. This plan should be based on several factors, such as the risk of stillbirth, risks associated with an additional blood sampling/IUT or the actual consequences of the anemia. All of these factors should be balancing against the risks of prematurity, morbidity of anemia and the risk of having hyperbilirubinemia prior to delivery[8].
Fig 2. Management of Rh D (–) women and fetuses at risk of anemia. GA= gestational age
3.7 OUTCOMES

Still today, there is a lack of information on neonatal outcomes and the frequency of complications of severely anemic fetuses that was treated by IUTs. Philip et al. reports a significant correlation between the severity of anemia and fetal outcome in Rh isoimmunised pregnancies, with the antenatal maternal serum IAT titer. Which makes it possible to express the severity, higher the antibody titer is and the more would the fetal severity be[16]. Overall perinatal mortality when severe fetal anemia is treated with IUTs, has decreased to <10%. Nowadays, postnatal manage is primarily centered on exchange transfusions to prevent kernicterus, and phototherapy for treatment of hyperbilirubinemia[8]. Tiblad et al. presented in 2011, a review of all the intravascular transfusion for red cell isoimmunisation over 20 years in Stockholm, Sweden. Where they reported the outcomes of 284 in utero transfusions in 86 pregnancies in 72 women. In >60% of the neonates, exchange transfusions were indicated and >97% required phototherapy. The newborns were also at risk for neonatal cholestasis, because of elevated levels of conjugated bilirubin[42].

The intrauterine blood transfusion is also related to maternal long-term outcomes, which is mainly associated with risk of immunization to additional antigens. In one large cohort study made by Schonewille et al. 25% of women formed additional antibodies following the IUTs, and postpartum more than 70% had multiple red cell antibodies[43].

Several studies have reported on the long-term outcomes of infants born after IUT. Where it is reported that main outcomes are related to neuro- and cardiovascular development. The LOng-Term follow- up after intra-Uterine transfusionS study (LOTUS) is today, the largest study to report the neurodevelopmental outcomes in children who suffered from hemolytic disease and was treated with IUT. The cohort study included 219 children, in the age of 2 to 17 over a 20-year period (1988-2018). The study reported an incidence of neurodevelopmental impairment more than 4% and in case of hydrops, the incidence increased progressively[44].

Wallace et al. provided the first evidence of altered cardiovascular development in humans that were exposed to fetal anemia and intrauterine transfusions. They proved in their study that adult survivors of fetal anemia and IUTs have a smaller and relatively increased walls of left ventricle, decreased myocardial perfusion at rest suggestive of coronary endothelial dysfunction and increased sympathetic tone[45].
CHAPTER 4: METHODOLOGY

This retrospective observational study was conducted at the Hospital of Lithuanian University of Health Sciences Kaunas Clinics, department of Obstetrics and Gynaecology. In our study we included all previous cases with IUT due to Rh isoimmunisation. A total number of 20 cases with IUT were included from January 1, 2004, through March 31, 2019. All relevant information about the antenatal course of these pregnancies, intrapartum data and immediate neonatal outcome was extracted from clinic files (Department of Obstetrics and Gynaecology). Necessary data on neonatal status were retrieved from the database of the Department of Neonatology.

A total of twenty intrauterine blood transfusions to ten pregnancies with Rh isoimmunisation were included to this study. Collected data was divided into several categories – maternal demographic data (age of mother, gestational weeks and antibody titer); fetal condition before IUT (MCA-PSV, SpA-PSV, pre-transfusion Hb and pre-transfusion Hct); fetal condition after IUT (post-transfusion MCA-PSV immediately after procedure and 24 hours after transfusion, post-transfusion SpA-PSV, post-transfusion Hb and Hct) and newborn status (gestational age at delivery, Apgar score 1 min, Apgar score 5 min, weight at birth (g) and number of times neonatal blood transfusion was performed after birth).

By using these parameters we aim to correlate increased MCA-PSV and SpA-PSV values with decreased fetal Hb values pre-transfusion, hence determine the value of MCA-PSV and SpA-PSV for the prediction of fetal anemia.

By comparing the statistical significance of MCA-PSV MoM and SpA-PSV MoM, we will determine which approach will have the best prediction of fetal anemia in Rh isoimmunised pregnancies. The purpose of evaluating MCA-PSV MoM, Hb and Hct immediately following intrauterine transfusion, is to establish the correlation between the changes in these parameters. Where the goal is to see an immediate decrease in MCA-PSV MoM and an increase in Hb and Hct.

Statistical analysis

Mean and standard deviation (SD) were calculated for continuous variables. Correlations were calculated with Spearman’s test. Multi variable analysis – Friedman’s Two-Way analysis and Wilcoxon Signed Ranks Test were used to evaluate associations between change in MCA-PSV MoM, MCA-PSV MoM 24 hours after IUT, SpA-PSV MoM, level of Hb and Hct before and after an intrauterine transfusion.

Statistical analysis was performed with SPSS (Statistical Package for Social Science, Microsoft Inc., Chicago, USA) software 23rd version. A probability value (p) of less than 0.05 was considered significant.
CHAPTER 5: RESULTS

The maternal demographic and clinical characteristics are shown in Table 1. The mean age and gestational age of the pregnant women were 31,3±3,5 years old (range, 26 to 39 years) and 27,6±3,0 weeks (range, 23 to 32 weeks), respectively. The average number of pregnancies of the studied women was 6,15±3,23. More detailed information regarding maternal data is displayed in Table 2.

<table>
<thead>
<tr>
<th>Table 2. The demographic data of the pregnant women</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>Gravida</td>
</tr>
<tr>
<td>Weeks of gestation</td>
</tr>
<tr>
<td>Valid N (listwise)</td>
</tr>
</tbody>
</table>

Over the 15-year study period, there were ten pregnancies where twenty IUT (n= 20) were performed. In four patients one transfusion, in four patients two transfusions and in two patient four transfusions at different gestational ages were conducted. In the rhesus-immunized fetuses, the mean pre-transfusion MCA-PSV was 1,84±0,46 MoM (n=20) and the SpA-PSV was 1,91±0,55 MoM (n=15), respectively. More detailed information regarding fetal condition before IUT is presented in Table 3. Of these twenty transfusions, eighteen had a MCA-PSV greater than 1,5 MoM and two lower than 1,5 MoM. However, in the two cases where MCA-PSV values pre-IUT did not exceed >1,5 MoM, signs of ascites was continuously present and they had previously received IUT.

<table>
<thead>
<tr>
<th>Table 3. Fetal condition before IUT</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Pre-Transfusion MCA PSV</td>
</tr>
<tr>
<td>Pre-Transfusion MCA MOM</td>
</tr>
<tr>
<td>Pre-Transfusion SpA-PSV</td>
</tr>
<tr>
<td>Pre-Transfusion SpA MOM</td>
</tr>
<tr>
<td>Pre-Transfusion Hb</td>
</tr>
<tr>
<td>Pre-Transfusion Hct</td>
</tr>
<tr>
<td>Valid N (listwise)</td>
</tr>
</tbody>
</table>
Immediately after the transfusion, the mean MoM of MCA-PSV was 1,13±0,22 (n=19), there was a statistically significant decrease between the pre- and post transfusion values (p<0,001). The difference in improvement between the mean MCA-PSV values before and after IUT are presented per case in fig. 3 (where the median line represents the median values for healthy non-Rh isoimmunised fetuses).

Measurements were also taken 24 hours after transfusion; the mean MCA-PSV was 1,45±0,21 MoM (n=14). By using Wilcoxon signed ranks test, the results displayed that MCA-PSV MoM statistically significantly (p<0,001) increases in a 24 hours period after IUT when compared with values that were obtained immediately after IUT.

The mean MoM of SpA-PSV post-transfusion was 1,42±0,23 (n=11) and there was a statistically significant decrease between the pre- and post-transfusion values (p<0,008). In table 4, there is more detailed information regarding fetal condition after IUT.

Fig 3. The mean MCA-PSV values before and after transfusions.
Table 4. Fetal condition after IUT

<table>
<thead>
<tr>
<th>Condition</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-transfusion MCA-PSV</td>
<td>19</td>
<td>28.00</td>
<td>67.50</td>
<td>40.5484</td>
<td>9.98635</td>
</tr>
<tr>
<td>Post-transfusion MCA MOM</td>
<td>19</td>
<td>0.80</td>
<td>1.66</td>
<td>1.1342</td>
<td>0.21940</td>
</tr>
<tr>
<td>Post-transfusion MCA-PSV 24h after transfusion</td>
<td>14</td>
<td>34.70</td>
<td>74.90</td>
<td>50.7479</td>
<td>10.59471</td>
</tr>
<tr>
<td>Post-transfusion MOM 24h after transfusion</td>
<td>14</td>
<td>1.08</td>
<td>1.84</td>
<td>1.4514</td>
<td>0.21483</td>
</tr>
<tr>
<td>Post-transfusion SpA-PSV</td>
<td>11</td>
<td>29.00</td>
<td>52.00</td>
<td>44.2091</td>
<td>6.82136</td>
</tr>
<tr>
<td>Post-transfusion SpA MOM</td>
<td>11</td>
<td>1.03</td>
<td>1.80</td>
<td>1.4173</td>
<td>0.23384</td>
</tr>
<tr>
<td>Post-transfusion Hb</td>
<td>15</td>
<td>64</td>
<td>170</td>
<td>113.60</td>
<td>29.323</td>
</tr>
<tr>
<td>Post-transfusion Hct</td>
<td>15</td>
<td>19.60</td>
<td>51.90</td>
<td>34.3333</td>
<td>8.93146</td>
</tr>
<tr>
<td>Valid N (listwise)</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There was a weak correlation ($r=-0.270$) between pre-transfusion values of MCA-PSV MoM and Hb, and the correlation between pre-transfusion values of MCA-PSV MoM and Hct was also weak ($r=-0.152$).

We could see a very strong correlation ($r=-0.850$) between pre-transfusion values of SpA-PSV MoM and Hb, which also presented statistically significant ($p<0.001$). Another very strong correlation ($r=-0.833$) is observed between pre-transfusion values of SpA-PSV MoM and Hct.

A strong correlation ($r=-0.609$) was observed between post-transfusion values of MCA-PSV MoM and Hb, which indicates an effective treatment. A strong correlation was established between MCA-PSV MoM and Hct ($r=-0.649$) post-transfusion in this study, which also indicates an effective treatment of the fetal anemia.

The correlation between post-transfusion SpA-PSV MoM and Hb was weak ($r=-0.217$) and weak correlation ($r=-0.233$) was also established between SpA-PSV MoM and Hct.

The mean pre-transfusion Hb and Hct were $50.47\pm 20.75$ g/l (range, 21 to 81 g/l) and $15.47\pm 6.05\%$ (range, 6.90 to 24.90\%), respectively. In post-transfusion values of Hb and Hct, the means were $113.60\pm 29.32$ g/l (range, 64 to 170 g/l) and $34.33\pm 8.93\%$ (range, 19.60 to 51.90\%), respectively. Furthermore, there was a statistically significant ($p<0.001$) increase between the pre- and post-transfusion values. In fig.4, Hb values pre-IUT and post-IUT are presented per case for the purpose of presenting the immediate results of IUT (where the median Hb represents the median values for healthy non-Rh isoimmunised fetuses).
The mean age at birth was 33.33±1.88 weeks of gestation. The mean weight at birth was 2217.6±568.13 grams. A mean number of blood transfusions made after delivery was 1.36±0.63. More detailed information regarding the status of newborn is displayed in Table 5.

**Table 5. Newborn status**

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age at delivery</td>
<td>18</td>
<td>30</td>
<td>35</td>
<td>33.33</td>
<td>1.879</td>
</tr>
<tr>
<td>Apgar 1 min</td>
<td>18</td>
<td>4</td>
<td>9</td>
<td>6.83</td>
<td>1.581</td>
</tr>
<tr>
<td>Apgar 5 min</td>
<td>18</td>
<td>5</td>
<td>10</td>
<td>7.94</td>
<td>1.392</td>
</tr>
<tr>
<td>Weight at birth in grams</td>
<td>20</td>
<td>1050</td>
<td>2935</td>
<td>2217.60</td>
<td>568.198</td>
</tr>
<tr>
<td>Number of blood transfusions</td>
<td>14</td>
<td>1</td>
<td>3</td>
<td>1.36</td>
<td>0.633</td>
</tr>
<tr>
<td>Valid N (listwise)</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER 6: DISCUSSION

Detecting the fetal anemia in Rh isoimmunised fetuses by non-invasively approach such as Doppler velocimetry has been used in praxis previously [1,2,22,24]. Regarding an establishment of most accurate diagnostic approach and the correlation between the change in Hb, Hct and immediate change in MCA-PSV, SpA-PSV post-IUT, would be useful for improving the diagnostic approach in these kinds of pregnancies and would also improve the outcomes of newborns. There are some studies that claims that MCA-PSV is a useful method in the evaluation of fetal anemia, this includes Zimmermann et al. which reports of a significant correlation between fetal anemia and increased values of MCA-PSV [21]. However the usefulness of SpA-PSV in evaluating fetal anemia is not particularly established, since studies are very limited regarding using SpA-PSV as a useful method.

In this retrospective observational study we conducted and analyzed the data to determine the value of MCA-PSV and SpA-PSV for the prediction of fetal anemia in Rh isoimmunised pregnancies, and to assess if these methods are useful to evaluate fetal anemia and which one is the better predictor. Also to determine the correlation between the change in MCA-PSV and SpA-PSV with the change in fetal Hb and Hct that occurs immediately following IUT.

Due to the fact that a worldwide screening program and prophylaxis for Rh isoimmunisation exists, there is a low incidence rate across the world and therefore most of the published articles include only a few clinical cases. This is what makes this study unique, despite that the sample size of the study is not big. We evaluated twenty transfusions during the study period. Our data demonstrated that in all twenty cases that were studied, the mean MoM of MCA-PSV was increased. The pre-IUT MCA-PSV was $>1.80$ MoM. Previous studies reports that MCA-PSV levels of $>1.5$ MoM is with certainty a good method for prediction of anemia in Rh isoimmunised fetuses where no IUT has been performed [1,46]. However, MCA-PSV values pre-IUT did not exceed $>1.5$ MoM in all of the twenty cases, but in these two cases that didn’t exceed $>1.5$ MoM signs of ascites was continuously present which is a sign that could indicate a severe anemia. Important facts in these two deviant cases are that they have previously received IUTs before the evaluation and it has been established that MCA-PSV after IUT is not so accurate. The MCA-PSV values were determined just after the IUT and the mean of MoM was decreased compared with pre-IUT. This shows us that by correcting the anemia with IUT, the MCA-PSV values quickly straighten out to lower ranges and therefore decreases possible complications and increases chances for good fetal outcome.

In all of the cases that were studied, the MCA-PSV MoM increases within 24 hours after IUT. One can argue that this provides us with information regarding most optimal timing for next transfusion. However, there has been a lot of discussion regarding however Doppler velocimetry can be a reliable method to predict fetal anemia after several IUT. Where numerous studies has been
conducted and they argue that MCA-PSV MoM can be trusted after IUT has been performed 2 to 3 times[25,47].

The pre-transfusion SpA-PSV was >1,90 MoM, which is a slightly higher value compared with MCA-PSV MoM. The mean MoM of SpA-PSV post-transfusion was decreased immediately after intrauterine transfusion compared with pre-IUT values. Unfortunately values were not obtained 24 hours after the transfusion, therefore there was no possibility to compare or correlate the measurements between the two arteries (MCA and splenic artery).

There is correlation between MCA-PSV MoM and SpA-PSV MoM and Hb pre-IUT. Stronger correlation is between SpA-PSV and Hb pre-IUT, which is interesting since it suggests that measurements of splenic artery are better in prediction of fetal anemia due to Rh isoimmunisation. It makes us question if not Doppler velocimetry of splenic artery should be the gold standard in the evaluation of fetal anemia. But at the same time, since we only included data that was obtained pre-IUT in this correlation, we therefore have a smaller sample size of SpA-PSV (n=9) compared to MCA-PSV (n=10), which makes it less reliable. A larger study needs to be conducted to evaluate reliability of these findings.

The expected Hb and Hct increase and MCA-PSV decrease immediately following the IUT was observed in all twenty cases that were studied, which indicates adequate and successful treatment. Similar findings have been observed in previously published studies[18,25,47]. By assessing the pre- and post-transfusion values of Hb and Hct together with the immediate change in MCA-PSV MoM, gives us the possibility to evaluate if the amount of transfused blood was adequate.

The correlation between post-transfusion SpA-PSV MoM and Hb was weak and weak correlation was also established between SpA-PSV MoM and Hct. Both of these two weak correlations could be due to lack of data, since in most of the cases SpA-PSV was not evaluated after IUT.

The nine out of ten newborn received one or multiple blood transfusions after delivery. The mean number of transfusions was more than one transfusion per newborn. The single newborn that did not received any transfusions, was treated with phototherapy instead.

The limitations of our study were its retrospective nature and the limited number of the anemic fetuses that were analyzed. The ability to draw conclusions about the findings obtained during the study is hampered due to the small number of transfusions, in a few cases we lack the pre-IUT values of SpA-PSV and the values MCA-PSV 24 hours after IUT, and in most cases SpA-PSV was not evaluated after IUT.

In summary, the correlation between MCA-PSV MoM, SpA-PSV MoM with fetal Hb indicates that both arteries are valuable parameter in predicting fetal anemia in Rh isoimmunised pregnancies. Where as in this study, the splenic artery is seen to be the better predictor. Rapid decreases in the MCA-PSV MoM and SpA-PSV MoM can be observed by Doppler ultrasound
conducted immediately after the IUT. However the mean of MoM MCA-PSV, although demonstrating a post-transfusion decrease, they were highly variable in the immediate post-transfusion period. And all of the cases showed an increase of MCA-PSV within the first 24 hours. It was not possible to determine any variability in SpA-PSV MoM 24 hours after transfusion, since these values were not obtained. The post-IUT Hb and Hct was significant increased compared with pre-IUT values in all cases that could be obtained.
CHAPTER 7: CONCLUSION

1. The peak systolic velocity of MCA measured by Doppler velocimetry and expressed in MoM, is a method for the prediction of fetal anemia in Rh isoimmunised pregnancies.

2. The Doppler velocimetry of splenic artery, expressed in MoM, is an efficient method for the prediction of fetal anemia in Rh isoimmunised pregnancies.

3. The Doppler value indices of MCA and splenic artery for the prediction of fetal anemia are relatively different. Where in this study, the SpA-PSV MoM is the better predictor of them two.

4. There is a strong correlation, which is also statistically significant between the changes in MCA-PSV MoM and fetal Hb immediately following IUT. Which shows us an increase in Hb value and a decrease in MCA-PSV MoM. However due to the lack of data in some cases, correlation between SpA-PSV MoM and fetal Hb is inconclusive.

5. There is a strong correlation, which is also statistically significant between the changes MCA-PSV MoM and fetal Hct immediately following IUT. Which shows us an increase in Hct value and a decrease in MCA-PSV MoM. However due to the lack of data in some cases, correlation between SpA-PSV MoM and fetal Hct is inconclusive.

6. In all of the cases, it is seen that the MCA-PSV MoM increases in a 24 hours period after IUT.
1. We suggest including Doppler evaluation of both MCA and splenic artery into the management guidelines of rhesus isoimmunised pregnancies.

2. Examination with Doppler velocimetry should be conducted to evaluate the presence and severity of fetal anemia. Especially focus on the velocimetry of splenic artery seeing that according to this study, it is a better predictor of fetal anemia in case of Rh isoimmunisation.

3. In order to obtain more accurate results in the future, the Doppler evaluation of both arteries should be performed pre-IUT, post-IUT and 24 hours after IUT.
REFERENCES


avoid administration of anti-D to pregnant women when the fetus is RhD-negative: Implementation in the NHS. BJOG An Int J Obstet Gynaecol. 2015;122(12):1682–6.


40. Bellone M, Boctor FN. Therapeutic plasma exchange and intravenous immunoglobulin as primary therapy for D alloimmunization in pregnancy precludes the need for intrauterine transfusion. Transfusion. 2014;54(8):2118–21.


