Immunohematological Surveillance of Rhesus D Negative Pregnancies in Under Equipped Area. About 34 Cases at Jason Sendwe General Hospital (Lubumbashi, Democratic Republic of Congo)

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Abstract

Background: The pregnancies of Rh D negative mothers are considered high risk as they can complicate maternal fetal alloimmunization and lead to hemolytic disease of the fetus and newborn. This pathology is grafted with a high morbidity, in its severe form we find anemia, jaundice and sometimes an anasarca. This is the most well-documented, best-known, and only incompatibility that benefits from preventive treatment. However, its global distribution is not homogeneous. It is more common in Caucasians than in blacks (15% of Rh D Neg/4%). Its diagnosis and management are at the forefront of technology in other areas. It should be noted that our country’s prevention policy is not at the forefront.

The objective of the study was to establish the immunohematological profile of Rh D allo immunizations in an under equipped environment.

Methodology: We conducted a cross-sectional and prospective descriptive study at Sendwe Hospital from July 27, 2016 to December 2017. The ABO/D blood type and the RH/Kell phenotype were sought. The search for irregular agglutinins (RAI) was carried out in gel technique, as well as an indirect tube coombs followed by an antibody identification in the mother. A direct coombs was carried out systematically at the Newborn.

Results: Among 470 pregnant women received 34 were negative Rh D 7.23%. Mothers were unaware of their negative D status in 70% of cases. The extended Rh phenotype is cc-ee in 70.59% of gestants. The majority were O blood type 58.3%, followed by 28.2% A, 8.3% AB and 4.2% B. All mothers were Duffy negative Fy (a-b-) (FY -1-2) and 94.12% Kell negative. Indirect tube coombs was positive in 76.47% of gestants and gel RAI in 20.59%. The antibodies identified with RAI are: Anti D (anti Rh1) and anti Cw (anti Rh8) not excluded in 71.4% of cases; Anti D and anti C (anti Rh2) in 28.6%. MHDN had manifested in 18 of the 26 pregnancies totally followed or 69.23%; and the direct coombs of these newborns was positive.

Conclusion: Rh D negative pregnancies are at-risk pregnancies. Their immuno-hematological monitoring allows the implementation of preventive measures for the detection of hemolytic disease of the newborn which is particularly severe in this blood group.
Keywords: Immuno hematology; Pregnancy Rh D negative; Alloimmunization; Hemolytic disease of the newborn; Lubumbashi; DRC

Introduction

Negative RH D maternal pregnancies are considered to be at high immunological risk as they may be complicated by alloimmunization feto maternal erythrocyte and induce hemolytic disease of the newborn [1-3].

In view of the high number of clinical manifestations and progressive severity with increasing number of pregnancies follow up protocols of Rh D negative mother have been established globally. In the absence of surveillance and preventive treatment, immunological accidents appear from the third pregnancy (single jaundice, moderate anemia or both). If no treatment is undertaken, subsequent pregnancies will be grafted with more severe immunological reactions: severe and early jaundice, early anemia, or both. These manifestations will evolve rapidly and be strictly fetal if no treatment is initiated: fetal antisera, repetitive miscarriages, fetal anemia, in utero death [3-5].

There are several protocols for tracking negative Rh D pregnancies that are continually updated based on technological advances. The first element of this surveillance includes knowledge of the Rhesus D negative blood group with blood type card in industrialized countries. The second element is the research of alloimmunization by RAI tube or gel or indirect coombs in gestants from the 16th week of amenorrhea that will be repeated at the 28th and 32nd weeks of amenorrhea. In third position the identification of the antigen and its titration to assess the severity of the immunization; in case of identification of the anti D and for titers greater than 1/32 anti D prophylaxis is recommended. In the presence of maternal genital bleeding, during pregnancy a Kleihauer will be performed to assess the severity of the hemorrhage and the passage of fetal red blood cells into the maternal circulation [5-8].

Materno fetal rhesus-type alloimmunization is the only one that benefits from preventive treatment administered from diagnosis. Many studies have been carried out around the world decrypting its pathophysiology, diagnosis and treatment. Currently the antenatal diagnosis is the most recommended because it allows a management centered on actually risky pregnancies. The advent of fetalm genotyping significantly reduced the number of mothers receiving rhogam without immunization. Fetal genotyping coupled with fetal Doppler monitoring has resulted in a significant decline in severe fetal manifestations such as anasarca, fetal anemia and fetal death in utero [3,9-12].

Once the diagnosis is made, the hematological immuno surveillance of the pregnancy is strengthened until the birth and allows to dictate the guidelines of the treatment:

- For severe alloimmunization during pregnancy with high titer, identification of anti-D and fetal anemia; the gestant will receive rhogam and transfusion or exchange transfusion in utero will be performed as often as necessary [5,9,13,14].
- For alloimmunization with low titers or fetal anemia within 72 hours of delivery Rhesogamma should be administered [5,9,15,16].
- The newborn should be treated based on the dominant sign of the newborn hemolytic disease. If jaundice is the dominant sign a conventional or intensive phototherapy should be carried out depending on the level of serum bilirubin and in severe cases a transfusion or exchange-transfusion. In case anemia is the predominant sign transfusion and/or exchange transfusion will be performed. In the presence of anasarca an exchange transfusion should be carried in urgency [17-19].

Data on hemolytic fetal and rhesus-type neonatal disease in Africa indicate a lower frequency of negative rhesus D in the black population 15%/4% [1,2,20]. Published studies of immunohematological surveillance of Rh D negative pregnancies indicate a lack of knowledge of Rh D negative status amongst healthcare workers and patients alike. Additionally, there is a knowledge gap on the implication of the disease on further pregnancies and standard guidelines are lacking [6,10,21-25]. The use of diagnostic, prevention and treatment techniques is highly uneven because of the disparity in the technical platforms of the institutions [13,16,26,27].

Africa’s birth and fertility rate are among the highest in the world and our country the DRC has an estimated rate of 6.2 pregnancies/woman; largely above the two pregnancies which may escape immunological manifestations. The aforementioned factors place the Congolese woman at risk as she delivers far more than two [6,24,28].

We justify the choice and interest of this study by:

The absence in our country of a protocol for immunohematological surveillance of negative Rh D pregnancies for the prevention of hemolytic disease in the newborn.

- Objectives of the study:
  - Define the immuno haematological profile of rhesus D type
  - Materno fetal allo immunization
  - Establish the evolutionary profile of negative Rh D pregnancies in underequipped environments
  - Determine the most appropriate diagnostic tests for under-equipped environments to develop a local immuno hematological surveillance protocol.

Methodology

We conducted a prospective, descriptive and cross-sectional
study at General Hospital Jason Sendwe from pre-natal consultation to Maternity from 24 July 2016 to 30 December 2017.

Our sample was of convenience and resumed all Rh D pregnancies in this study period, 34 negative Rh D pregnancies among 470 gestants.

❖ The inclusion criteria were:

- Rhesus D negative blood type, pregnant over 16 weeks of amenorrhea at pre-natal consultation at Jason Sendwe General Hospital
- Attended at least one information session on blood type and MFI and HDFN
- Freely agree to participate in the study
- The criteria for not inclusion were:
  - Rhesus D positive blood type
  - Refusal to participate in the study

Rh D negative blood pooling was retained after pooling by Beth Vincent’s method the presence of alloimmunization was retained on the basis of a positive indirect coombs and confirmed by the identification of a D antigen during gel irregular antiglobulin research. Hemolytic disease of the fetus and newborn was confirmed by the clinic and the positive direct coombs.

Results

Frequency of Rhesus D Negative Gestants (Figure 1)

Figure 1: Frequency of Rhesus D Negative Gestants Rh D negative gestants make up 7% of our study population.

a. Maternal Profile (Table 1).
b. Maternal Antecedent

i. Immunization Pathway (Figure 2)

![Immunization Pathway](image)

**Figure 2**: Immunization Pathway.

- Miscarriages and curettages were the most common routes of allo immunization.
- Previous newborns had more jaundice than anemia.
- Our gestants had in 23% an experience of fetal death in utero/early neonatal death.

ii. Blood Group Knowledge and Prophylaxia Use (Figure 3)

![Blood Group Knowledge and Prophylaxia Use](image)

**Figure 3**: Knowledge of the Blood Group and Use of Rhesogamma.

- None of them had a blood type card.
- Negative Rh D status was ignored in 44.12% of cases.
- Only 3% of this population had ever used rhogam.
Mapping of Other Blood Groups in Our Population

c. ABO

Group O was the most recovered 65%; followed by group a 24 % (Figure 4).

![Figure 4: Distribution of Subjects According to the ABO Group.](image)

Figure 5: Mapping Of Rh 2, Rh 3, Rh 4, Rh 5 Kell and Duffy.

Our sample expresses:

- Very weakly RH3 2.94% and Kell 5.88%, weakly RH2 26.47%
- Not at all the Duffy 0%
- And totally the RH4 100% and the RH5 100%

![Figure 5: Mapping Of Rh 2, Rh 3, Rh 4, Rh 5 Kell and Duffy.](image)
e. Extended Rhesus Phenotype
Table 2 Extended Rhesus Phenotype

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>subjects</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>cc-ee</td>
<td>24</td>
<td>70.59%</td>
</tr>
<tr>
<td>Cc-ee</td>
<td>9</td>
<td>26.47%</td>
</tr>
<tr>
<td>cc-Ee</td>
<td>1</td>
<td>2.94%</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 2: The most found phenotype is the cc-ee in 70.59% of cases.

f. Frequency Of Immunization In Indirect Coombs (Table 3)

<table>
<thead>
<tr>
<th>indirect coombs</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>8</td>
<td>23.53%</td>
</tr>
<tr>
<td>+</td>
<td>7</td>
<td>20.59%</td>
</tr>
<tr>
<td>++</td>
<td>12</td>
<td>35.29%</td>
</tr>
<tr>
<td>+++</td>
<td>2</td>
<td>5.88%</td>
</tr>
<tr>
<td>++++</td>
<td>5</td>
<td>14.71%</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>100.00%</td>
</tr>
</tbody>
</table>

Table 3: The immunization rate of pregnant women in our study was 76.47%; i.e. 3/4 of pregnant women.

g. Alloimmunization and Irregular Agglutinin Research
Table 4 Frequency of Immunization in Irregular Agglutinin Research

<table>
<thead>
<tr>
<th>IAR LIEGE</th>
<th>Frequency</th>
<th>percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEG</td>
<td>27</td>
<td>79.41%</td>
</tr>
<tr>
<td>POS</td>
<td>7</td>
<td>20.59%</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>100.00%</td>
</tr>
</tbody>
</table>

Table 4: Out of our 34 pregnant women; 7 had a positive irregular agglutinin test, i.e. 20.59%

Table 5: All negative indirect coombs were found to be negative in IAR as well.

<table>
<thead>
<tr>
<th>IAR</th>
<th>Positive Irregular agglutinin research</th>
<th>negative Irregular agglutinin research</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>positive indirect coombs</td>
<td>7</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Negative indirect coombs</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>7</td>
<td>27</td>
</tr>
</tbody>
</table>

Table 6: We had a high proportion of positive indirect coombs compared to positive Irregular agglutinin search and antigens identified either 19/26 or 73% and 100% of irregular agglutinin search were also positive in Indirect coombs

We noticed that in 100% of cases negative indirect coombs gave also negative irregular agglutinin search.

Antibody Identification

The alloimmunization rate in this population of Rh D negative pregnant women is 20.59%.

We have identified anti-D in 7 sera. All anti D were accompanied by a second antigen; the Anti D and Anti Cw combination were the most common 71% Figure 6.

Figure 6: Identified Antibodies.

Evolution of Pregnancies

Out of the 34 pregnant women followed; we have the course of 26 pregnancies; i.e. 76.47% compliance.

Hemolytic disease of the fetus and newborn was noted in 62% of cases figure 7 and tables 7-9.
Figure 7: pregnancy outcome.

<table>
<thead>
<tr>
<th>Positive HDFN</th>
<th>Negative HDFN</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive indirect coombs</td>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td>Negative indirect coombs</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>TOTAL</td>
<td>16</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 7: Sensitivity and Specificity of Indirect Coombs in HDFN.
SENSITIVITY = positive test / HDFN (+) = 16/16 = 100%
SPECIFICITY = negative test / HDFN (-) = 4/10 = 40%
The indirect coombs is more sensitive than specific.

<table>
<thead>
<tr>
<th>Positive HDFN</th>
<th>Negative HDFN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Irregular agglutinin research</td>
<td>6</td>
</tr>
<tr>
<td>Negative Irregular agglutinin research</td>
<td>10</td>
</tr>
<tr>
<td>TOTAL</td>
<td>16</td>
</tr>
</tbody>
</table>

Table 8: Sensitivity and Specificity of Irregular Antigens Search.
SENSITIVITY = 6/16 = 37.5%
SPECIFICITY = 9/10 = 90%

Irregular agglutinin research is more specific than sensitive in our study population.

<table>
<thead>
<tr>
<th>Gravidity</th>
<th>Positive Indirect Coombs</th>
<th>Positive Irregular Agglutinin Search</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRIMIGEST</td>
<td>1/3 (33.3%)</td>
<td>0/3 (0%)</td>
</tr>
<tr>
<td>PAUCIGEST</td>
<td>2</td>
<td>4/6 (66.6%)</td>
</tr>
<tr>
<td>3</td>
<td>6/7 (85.7%)</td>
<td>0/7 (0%)</td>
</tr>
<tr>
<td>MULTIGEST</td>
<td>4</td>
<td>2/2 (100%)</td>
</tr>
<tr>
<td>5</td>
<td>4/4 (100%)</td>
<td>1/4 (25%)</td>
</tr>
<tr>
<td>6</td>
<td>3/4 (75%)</td>
<td>1/4 (25%)</td>
</tr>
<tr>
<td>LARGE MULTIGEST</td>
<td>7</td>
<td>2/4 (50%)</td>
</tr>
<tr>
<td>8</td>
<td>1/1 (100%)</td>
<td>1/1 (100%)</td>
</tr>
<tr>
<td>9</td>
<td>1/1 (100%)</td>
<td>0/1 (0%)</td>
</tr>
<tr>
<td>10</td>
<td>1/1 (100%)</td>
<td>0/1 (0%)</td>
</tr>
<tr>
<td>12</td>
<td>1/1 (100%)</td>
<td>0/1 (0%)</td>
</tr>
</tbody>
</table>

Table 9: Relation between Indirect Coombs, Irregular Agglutinin Search and Pregnancies.

In our study population, no RAI was positive in prim gravidae and anti-D alloimmunization was more noted in multigestes followed by paucigestics. Only one large multigest presented anti-D. The indirect coombs test found alloimmunizations of prim gravidae to large multigestes. Multiparas and large multiparas are the most immune Table 10.
Table 10: Relationship between Parity, Indirect Coombs and Positive Irregular Agglutinin Search.

<table>
<thead>
<tr>
<th>Parity</th>
<th>Positive Indirect Coombs</th>
<th>Positive Irregular Agglutinin Research</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primiparous</td>
<td>2/4 (50%)</td>
<td>1/4 (25%)</td>
</tr>
<tr>
<td>Paucipara</td>
<td>4/6 (66.6%)</td>
<td>2/6 (33.3%)</td>
</tr>
<tr>
<td></td>
<td>5/6 (83%)</td>
<td>0/6 (0%)</td>
</tr>
<tr>
<td></td>
<td>7/7 (100%)</td>
<td>2/7 (28.2%)</td>
</tr>
<tr>
<td>Multiparous</td>
<td>1/1 (100%)</td>
<td>1/1 (100%)</td>
</tr>
<tr>
<td></td>
<td>3/5 (60%)</td>
<td>1/5 (20%)</td>
</tr>
<tr>
<td></td>
<td>2/3 (66%)</td>
<td>0/3 (0%)</td>
</tr>
<tr>
<td>Large</td>
<td>2/2 (100%)</td>
<td>0/2 (0%)</td>
</tr>
<tr>
<td>Multiparous</td>
<td>2/2 (100%)</td>
<td>0/2 (0%)</td>
</tr>
</tbody>
</table>

The indirect coombs test reveals high rates of alloimmunization of primiparous to large multiparous. On the Irregular agglutinin research side, the anti-D alloimmunization rate is zero from parity 6 (Figures 8-11).

Use of Anti D Prophylaxis

- HDFN was present in 16 cases on 26 61.53%. Neonatal jaundice was the most common manifestation.

j. Neonatal Blood Group Rhesus D

Figure 8: In our population, 31% of subjects did not need prophylaxis because they were not immunized; 31% received rhogam and 34% refused.

i. Hemolytic Disease of the Newborn

Phototherapy was the most common treatment.
Among our population of newborns 96% had D Antigen (Figure 12).

![Figure 12: Neonatal Extended Rhesus, Kell and Duffy.](image)

The newborns in our study all express Rh 4 and Rh5, i.e. 100%; very little Rh 2 and Rh 3 25% and 12.5% respectively and not Kell and Duffy at all, i.e. 0% (Tables 11-14).

**Direct Coombs**

<table>
<thead>
<tr>
<th>Direct Coombs</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>18</td>
<td>75%</td>
</tr>
<tr>
<td>Negative</td>
<td>6</td>
<td>25%</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>100%</td>
</tr>
</tbody>
</table>

**Table 11**: Coombs direct was positive in 75% of our newborn.

**Direct Coombs and HDFN**

<table>
<thead>
<tr>
<th>Direct Coombs and HDFN</th>
<th>Sick</th>
<th>No Sick</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>14</td>
<td>4</td>
<td>18</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>10</td>
<td>24</td>
</tr>
</tbody>
</table>

**Table 12**: In our population study; direct antiglobuline test is more sensitive than specific.

Sensibility=14/14 = 100%
Specificity= 6/10= 60%

**Diagnostic**

**Maternal Fetal Incompatibility**

<table>
<thead>
<tr>
<th>Maternal Fetal Incompatibility</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rh D And Rh Cw</td>
<td>5</td>
<td>14.7%</td>
</tr>
<tr>
<td>Rh D And Rh C</td>
<td>2</td>
<td>5.8%</td>
</tr>
<tr>
<td>None</td>
<td>8</td>
<td>23.5%</td>
</tr>
<tr>
<td>Abo</td>
<td>19</td>
<td>56.0%</td>
</tr>
</tbody>
</table>

**Table 13**: In our population, Rh D foeto maternal incompatibility represents 20.5%. The first cause was ABO incompatibility 56%.

**Treatment of Newborn**

<table>
<thead>
<tr>
<th>Treatment of Newborn</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phototherapy</td>
<td>14</td>
<td>58.3%</td>
</tr>
<tr>
<td>Transfusion</td>
<td>7</td>
<td>22.7%</td>
</tr>
<tr>
<td>Exchange Transfusion</td>
<td>2</td>
<td>8.3%</td>
</tr>
<tr>
<td>None</td>
<td>10</td>
<td>41.6%</td>
</tr>
</tbody>
</table>

**Table 14**: Treatment of new born.
Discussion

Frequency

Our negative Rhesus D frequency is 7.23%; this frequency is:

- Higher than that found in Congo in general: 3% at Kabemba in 2017, 1.6% in 2016 and at assumani2.3% [29-31]. This difference can be explained in part by the difference in the samples.
- Within the upper limits of the Black race 5-7.8% [20];
- However, our result is much lower than the Maghreb data where percentages of 11 to 30% are observed [10,32,33].
- This is also justified by the type of population and different samples as mentioned above.

Knowledge of blood type

- In our series, less than half of pregnant women knew their blood type 44.12% and just over half that of their spouses 55%. No gestante was in possession of a blood grouping card because our national blood transfusion policy does not require it. These rates reflect transfusion safety in the Congo and Southern Sahara [27,34-36].
- These rates are lower than those of Naimi in Morocco and Manon in Belgium; in their studies all pregnant women held a blood type card [9,10].

Mapping of maternal blood groups

- In ABO, the proportions are close to those observed in other studies with a predominance of O; however, the proportion of subject AB is higher than the data known for the breed and that of subject B very low.
- For rhesus antigens there is a higher proportion of RH2 positive subjects than in the other populations studied. The remaining antigens have a distribution similar to the known data for race and country [1,20,35,37,38].
- The most common rhesus phenotype is cc-ee; it is the most common rhesus negative phenotype of the black population [1,20,37-39].

Allo immunization pathway

- More than a third of our population 38% had a history of abortion; then there were curettages 28% and previous transfusions 5.88%. In his study Ngo Sack in Yaounde, Cameroon, found a higher proportion of previous transfusion history [40].
- These different histories of miscarriages constitute both probable routes of allo immunization and signs of allo immunization foeto maternal [5,11,41].

Allo immunization

- We found high immunization rates in Rh D-negative mothers.
- With indirect coombs, we found 76.4% of immunized women or 3/4 of our subjects. The explanation could be:
- In the type of alloimmunization diagnosed with indirect tube coombs; this examination is by no means specific to D immunization. The population of our study is more exposed to ABO-type MFI due to its blood group distribution [42-45].
- In a high proportion of multigest and large multigest 12/34 or 35%, and each pregnancy is a new risk of immunization [11,18].
- We noted that the immunization threshold for coombs rose with the number of pregnancies in our series; indeed, a greater proportion of positive indirect coombs was observed in multiparas. It also appeared that the indirect coombs was positive even in primigests.
- The Irregular agglutinin research has given us an immunization rate of 20%, which is significantly higher than that of all the studies consulted:
- Literature data for industrialized countries place this frequency between 0.5 and 1% [2,46]
- By compiling different studies, our rate is twenty times higher than countries that have excellent hematological immunosurveillance of rhesus pregnancies, and almost double that of other low-income countries:

<table>
<thead>
<tr>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Country</td>
<td>France</td>
<td>Canada</td>
<td>Australia</td>
<td>Holland</td>
<td>Morocco</td>
<td>Turkey</td>
<td>Tunisia</td>
<td>Bangladesh</td>
</tr>
<tr>
<td>Rate</td>
<td>1,43% and</td>
<td>0.54 à 0,81%</td>
<td>0,73%</td>
<td>1,23%</td>
<td>6%</td>
<td>8,74%</td>
<td>5,17%</td>
<td>5,14% to 15%</td>
</tr>
</tbody>
</table>
It appears that our population is more allo-immunized in Rh D than populations with higher proportions of D-negative subjects due to the lack of immuno-hematological monitoring. This is in line with Ralli’s claims in his study on the dangerousness of the lack of prevention of allo-immunization, which found more severe hemolytic diseases than in the monitored population [26].

All gestants in our study with positive Irregular agglutinin research had two antigens. This finding was not echoed in the other studies. We found anti-D combined with other antigens in other studies; but not 100% as in our population [11,13,46,53].

We also noted that RH8 a rare antigen was most found coupled with anti D in 71.4% of cases, this rate is one of the highest in the literature. Paci found it in 3.3%; Pal 0.6% and Koelwijn 1.65% [46,48,49]. These frankly different distributions are probably justified by the polymorphism of blood groups and the transfusion safety inherent in each country.

We found a specificity of 100% for the indirect coombs and a sensitivity of 40% while it was the opposite at the level of the Irregular agglutinin research that was more sensitive 90% than specific. Other studies around the world have looked at diagnostic methods by comparing them and each result depends on the population studied and the technique used [13,54,55] in our series we translate that a well conducted indirect coombs test in under equipped environment makes it possible to diagnose a feto maternal allo immunization regardless of the blood group concerned and that the Irregular agglutinin research is more specific to the type Rhesus.

It is known that indirect coombs diagnoses allo immunizations in all blood groups systems without distinctions; while gel Irregular agglutinin research does not diagnose allo immunizations of type ABO [2,43,56,57]. Our study population is more exposed to allo immunizations of type ABO and these results confirm that the tube indirect ant globulin test is applicable in Africa and will make it possible to diagnose feto maternal allo immunization. Bigot stipulated that this test only worked for the rhesus and did not allow the diagnosis of feto maternal allo immunization in ABO; contrary to the use of papain [58]. It is possible that this is a difference of technique because in an equipped environment they have coombs washers that automatically wash red blood cells three times. In the absence of coombs washers, this wash must be performed by the lab technician by hand. We noted the absence of this step on local laboratory protocols.

Gel Irregular agglutinin research was more specific (90%) than sensitive (37.5%). The low sensitivity of Irregular agglutinin research can be explained by the type of population present, ours being more at risk of developing hemolytic disease of type ABO it is obvious to have a negative Irregular agglutinin research even in the presence of hemolytic disease of the newborn.

These results allow us to highlight the preponderance of allo immunizations of type ABO over the type Rhesus D. Other authors found that an ABO incompatibility delayed the appearance of allo immunization of type Rhesus [42,43,56,57,59].

Allo immunization and obstetric identity

It is classically known that Rhesus incompatibility does not manifest in primiparous unless they have been previously immunized by transfusion.

We noted a positive indirect coombs in a primigest of our study while the Irregular agglutinin research was negative. In ABO type allo immunization, immunization may appear from the first pregnancy.

While positive indirect coombs were noted from primiparous to multiparous; the RAI which is the gold standard of allo immunization Rhesus has turned negative in gestants of more than 6 pregnancies. We found no explanation for this lack of immunization and make some assumptions:

Were these pregnant women weak D or partial D? It is possible in this case for a negative RHD subject not to develop immunization when coming into contact with positive RHD red blood cells. During the colonial period Lambotte had found a low proportion of D in the Congolese population at the time. We did not conduct research on low D in our population [2,9,38,60,61].

Would ABO incompatibility have been a protective factor for Rhesus incompatibility? It is known that ABO antigens appear earlier in fetal red blood cells than in the Rhesus. Thus, in case of incompatibility ABO and Rhesus, this first takes precedence over the other and the recognition of Rhesus D is delayed or decreased [1,42,56,62].

Did she have negative Rh D pregnancies before this one? It is not possible for a negative RHD subject to be immune to Do if all pregnancies are also negative RHD [2,9,21]. Unfortunately, we were unable to perform the blood grouping of the siblings in this case.

These questions leave an opening for further studies on the Rhesus D of Congolese subjects in our environment, especially as its composition is dynamic, without forgetting the phenomenon of intermingling.

Hemolytic disease of the newborn

The percentage of newborn immunized is 18/24 or 75%; it should be noted that two pregnancies resulted in a miscarriage. This rate is higher than that found in the literature 15-25%. It should be noted that the antigenic profile of our gestants is strongly O and that they are all rhesus negative for a majority of rhesus D positive neonates. In addition, the lack of immune hematologic surveillance of pregnancies and their high rate of pregnancies may justify this high frequency.

Our positive direct coombs rates are higher:
- To those found in Yaounde by Sock who noted a low
positivity rate of coombs direct in his population of newborn 2/34 or 5.88% [63]. This difference is probably related to the difference in population and direct coombs technique because despite these low levels of direct coombs it isolated hemolysis in sera.

- To those of Bel Hadj in Tunisia 21.42% with signs of hemolysis related to hemolytic disease present even for coombs negative [64].

- To those of Monica Kapassa in Zambia 97/349 newborns or 27.8% for manifestations of hemolytic disease even on direct negative coombs [57].

All immunized neonates had hemolytic disease of the newborn; however, the proportion related to the rhesus type was 29%; the other neonates likely developed hemolytic disease of the ABO type. This finding, corroborates that ABO incompatibility is the first in terms of frequency and that it is the most common incompatibility found in black Africans [5,7,42,46,59,63,65]. We were able to note that 8 newborns did not have hemolytic disease of the newborn despite the mother being RhD negative.

The presence of maternal allo-immunization does not necessarily lead to hemolytic disease of the newborn because the newborn may not be a carrier of the gene; one of our subjects did not develop hemolytic disease of the newborn despite identified antibodies.

**Treatment**

The management of our newborns was carried out in the neonatal unit of the hospital Sendwe.14 newborns benefited from conventional phototherapy either 58.3%; 7 were transfused or 29.7% or 2 were exsanguinated or 8.33%.

Our rates of phototherapy and exchange transfusions are lower than those of Imen Bel Hadj in Tunisia in 2019 (77.5% and 13.3%), while our rates of transfusions are higher than his 9.1% [64].

Our care is similar to that of Mutombo at the Bonzola Hospital in Mbuji Mayi in DR Congo, but our figures are different; in fact he resorted to transfusion in 30% of cases, exchange transfusion in 24% and phototherapy was indicated in 5% of cases [22].

There is a big difference compared to the LOTUS study carried out in the Netherlands by Smits Wintjens which included besides phototherapy, transfusion and exchange transfusion; the use of albumin, intensive phototherapy and intravenous immunoglobulins. His study also included antenatal management by transfusion in utero [19].

These differences in support are related to the type of sample and the technical tray of each unit.

**Neonatal outcomes**

The evolution of our newborns was good 91.2% of our subjects left the hospital (87.5% healing without sequelae and 4.2% left with sequelae); this rate is similar to that of Mutombo in Bonzola 82.3% in the province of Kasai Oriental [22].

**Conclusion**

The clinical profile of our allo immunizations revealed an allo immunizations of 76.47% of gestants in all groups and in Rhesus of 20%; the anti-D was accompanied by another antibody in 100% of cases and hemolytic disease of the newborn occurred in 62% of cases. The phenotype of our group is cc-ee in the vast majority of cases.

The development of pregnancies was favorable in 84.7% of cases, jaundice was the most found manifestation. Phototherapy was the most widely used treatment, followed by transfusion and exsanguinous-transfusion.

There is no immune hematological monitoring protocol for negative Rh D pregnancies in our country. It would be wise to proceed with extensive blood grouping in rhesus of all gestants with distribution of blood group map. The indirect tube coombs test achievable in our laboratories had a 100% sensitivity and the gel Irregular agglutinin research a specificity of 90%. It is therefore perfectly possible to follow up with the indirect coombs test. A positivity of this test should give rise to the prescription of rhesogamma during pregnancy to allow a split purchase of this molecule whose cost is not within the reach of the Congolese. The treatment of hemolytic disease of the newborn will depend on the predominant manifestation.

Immunohematological monitoring of negative Rh D pregnancies in an under-equipped environment is feasible with the establishment of a multidisciplinary team.

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**References**


47. Paci C (2017) État des lieux et suivi de l’allo-immunisation anti-érythrocytaire des femmes enceintes, par l’EFSAM de 2006 à 2016 To cite this version : HAL Id : dumas-01609299.
58. Monica K (2012) Hemolytic Disease of the Newborn among Newborn Babies with ABO Incompatibility , at the University Teaching Hospital, Lusaka, Zambia.