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Immunization to red blood cell antigens in patients with sickle cell disease in Martinique Island – French West Indies
ETHNIC POPULATION BACKGROUND IN FRANCE

- **France:** 66 million inhabitants
- **Ethnically mixed population**
  - Migrant people from the former French colonies of Africa (1\textsuperscript{st} or 2\textsuperscript{nd} generation)
  - Overseas territories
THE FRENCH OVERSEAS TERRITORIES
THE WEST INDIES AREA

“West Indies” area
Islands of the Caribbean sea
THE MARTINIQUE ISLAND

~7000 kms
✈️ 8h30
Discovered in 1502

Massive increase of the population in the 17th and 18th century due to the incoming slaves from tropical and equatorial Africa for sugar cane production
POPULATION BACKGROUND

Fy(a-b-)
HrS- (RH:-18)
HrB- (RH:-34)
Sec- (RH:-46)
CEST- (RH:-57)
CELO- (RH:-58)
CEAG- (RH:-59)
CEVF- (RH:-61)
U-
U+ \text{var}
Js(b-)
At(a-)
...
HgbS

Martinique Island

Transatlantic slave trade

~400,000 inhabitants today
>85% are of African ancestry
SICKLE CELL DISEASE IN MARTINIQUE ISLAND

• ~1,500 patients, including 250 children
• 40,000 inhabitants (8-10%) are heterozygous for HgbS
• Systematic HgbS screening since 1984: 5-10 homozygous neonates/year (1/300 births/year)
High rate of blood donors of African ancestry in Martinique (~95%)

=> Almost all SCD patients are transfused in an intra-ethnic background
AIMS OF THIS STUDY

To determine whether the frequency of RBC alloimmunization in SCD patients of Martinique Island would be significantly lower, as donors and patients are almost all from the same ethnic background.
351 SCD (SS) patients’ records retrospectively investigated

All patients were serologically matched for C, E, c, e, K antigens

Additional serological matching for Fy^a/Fy^b, Jk^a/Jk^b, S/s in case of alloimmunization to any RBC antigen

60% of patients transfused more than 3 times and 11% chronically transfused

No patients transfused with genotyped blood donors
## RESULTS

<table>
<thead>
<tr>
<th>ABO/D</th>
<th>SCD patients from Martinique</th>
<th>Blood Donors from Martinique</th>
<th>General population in continental France</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>48.8</td>
<td>54</td>
<td>43</td>
</tr>
<tr>
<td>A</td>
<td>29.4</td>
<td>27.3</td>
<td>45</td>
</tr>
<tr>
<td>B</td>
<td>17.8</td>
<td>15.5</td>
<td>9</td>
</tr>
<tr>
<td>AB</td>
<td>4</td>
<td>3.2</td>
<td>3</td>
</tr>
<tr>
<td>D+</td>
<td>93</td>
<td>88</td>
<td>85</td>
</tr>
<tr>
<td>D-</td>
<td>7</td>
<td>12</td>
<td>15</td>
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<tr>
<td>Rh</td>
<td>SCD Patients from Martinique</td>
<td>Blood Donors from Martinique</td>
<td>General population in continental France</td>
</tr>
<tr>
<td>-----------------------------------------</td>
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<td>-------------------------------</td>
<td>------------------------------------------</td>
</tr>
<tr>
<td>D+C-E-c+e+ (R₀R₀)</td>
<td>53</td>
<td>44.7</td>
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</tr>
<tr>
<td>D+C+E-c-e+ (R₁R₁)</td>
<td>2.6</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td>D+C-E+c+e-</td>
<td>0.3</td>
<td>1.1</td>
<td>2</td>
</tr>
<tr>
<td>D+C+E+c+e+</td>
<td>2.6</td>
<td>5.7</td>
<td>14</td>
</tr>
<tr>
<td>D+C+E-c+c+e+</td>
<td>24.7</td>
<td>28.8</td>
<td>35</td>
</tr>
<tr>
<td>D+C-E-c+c+e+</td>
<td>14.3</td>
<td>13.4</td>
<td>12</td>
</tr>
<tr>
<td>Blood type</td>
<td>SCD patients from Martinique</td>
<td>Blood Donors from Martinique</td>
<td>General population in continental France</td>
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<td>------------</td>
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<td>------------------------------------------</td>
</tr>
<tr>
<td>Jk(a-b+)</td>
<td>9.5</td>
<td>9.3</td>
<td>23.4</td>
</tr>
<tr>
<td>Jk(a+b+)</td>
<td>45</td>
<td>41.5</td>
<td>50.3</td>
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<tr>
<td>Jk(a+b-)</td>
<td>45.5</td>
<td>49.2</td>
<td>26.3</td>
</tr>
<tr>
<td>Fy(a-b+)</td>
<td>17</td>
<td>26.6</td>
<td>34</td>
</tr>
<tr>
<td>Fy(a+b-)</td>
<td>15.3</td>
<td>18</td>
<td>17</td>
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<tr>
<td>Fy(a-b-)</td>
<td>63</td>
<td>46</td>
<td>Rare</td>
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<tr>
<td>S-s+</td>
<td>65.3</td>
<td>64</td>
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<tr>
<td>S+s-</td>
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<td>7.5</td>
<td>11</td>
</tr>
<tr>
<td>S+s+</td>
<td>24</td>
<td>27</td>
<td>44</td>
</tr>
<tr>
<td>S-s-</td>
<td>1.8</td>
<td>2</td>
<td>Exceptional</td>
</tr>
</tbody>
</table>

⇒ The major blood phenotype frequencies appear to be very close between SCD patients and blood donors
⇒ Transfusion mostly in an intra-ethnic background
RESULTS

• Global immunization rate (auto/allo): 29%
• 27% if autoantibodies excluded
• 20% if likely naturally-occurring antibodies excluded (e.g. anti-M, anti-Le^a)
• 201 alloantibodies found, with 32 different specificities
• Anti-D, anti-C and anti-E represent 22% of all alloantibodies
<table>
<thead>
<tr>
<th>System</th>
<th>Alloantibody specificities</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rh (32.3%)</strong></td>
<td>D (7), C (21), E (17), V/VS (7), Cw (6), ce (2), Ce (3), Dw (1), Rh32 (1), hrS (1), Goa (1)</td>
</tr>
<tr>
<td>Kell (5.5%)</td>
<td>K (5), Kpa (4), Js (2)</td>
</tr>
<tr>
<td>Duffy (8.5%)</td>
<td>Fa (14), Fy3 (2), Fy5 (1)</td>
</tr>
<tr>
<td>Kidd (6.5%)</td>
<td>Jkb (12); Jka (1)</td>
</tr>
<tr>
<td><strong>MNS (22.4%)</strong></td>
<td>M (17), N (2), S (25), s (1)</td>
</tr>
<tr>
<td>Lewis (17%)</td>
<td>La (24), Leb (10)</td>
</tr>
<tr>
<td>H (1.5%)</td>
<td>Hi</td>
</tr>
<tr>
<td>Knops (1.5%)</td>
<td>Knops of undetermined specificity</td>
</tr>
<tr>
<td>Lutheran (0.5%)</td>
<td>Lu a</td>
</tr>
<tr>
<td>Dombrock (2%)</td>
<td>Doa (1), Dob (3)</td>
</tr>
<tr>
<td>Cartwright (1%)</td>
<td>Ytb (2)</td>
</tr>
<tr>
<td>Unidentified Ab to a high-prevalence antigen</td>
<td>2</td>
</tr>
<tr>
<td>Unidentified Ab to a low-prevalence antigen</td>
<td>1</td>
</tr>
</tbody>
</table>
DISCUSSION

• Why so many cases of Rh immunization, since blood transfusion is systematically performed with RBC units that match the C, E, c, e antigens?

• Why such a frequency of anti-S?
Partial antigens are frequent in SCD patients (Noizat-Pirenne Transfus Clin Biol 2012)

• ~7% of D+ are partial D
• ~30% of C+ are partial C
• ~2% of e+ are partial e

=> Possible Rh alloantibodies stimulated by RBC transfusion or pregnancies in female SCD patients
UNEXPECTED RATE OF RH ANTIBODIES

- Anti-E are often Rh autoantibodies, either found in E+ or E- patients. If found in a E- patient (common blood type), it mimics an alloantibody => “mimicking alloantibodies” Transfusion stimulates production of RBC autoantibodies!

- Weakly expressed Rh variants undetected in donors, especially C => Typical example of a potential missed C reactivity is the (C)ceS haplotype
(C)ceS type 1 haplotype

RHD

RHD*DIIIa-CE(4-7)-D
No D
Weak and partial C (MS24 clone)

RHCE

RHCE*ceS or RHCE*ce48C,733G,1006T
partial c
partial e
Loss of the HrB (RH34) high-prevalence antigen

No C at the genetic level but weak C reactivity (~2+) with anti-C MS24 clone (most commonly used anti-C reagent)
(C)ceS type 2 haplotype

RHD

RHD*D-CE(4-7)-D
No D
Very weak and partial C
(MS24 clone)

RHCE

RHCE*ceS or RHCE*ce48C,733G,1006T
Partial c
Partial e
Loss of the HrB (RH34) high-prevalence antigen

Less frequent than (C)ceS type 1 but at much higher risk of being mistyped as C-, while potentially responsible for C alloimmunization
A significant part of anti-S in S-s+ patients of African origin actually corresponds to weak auto-anti-U (cold IgG) (Janvier D & al. *Vox Sang* 2002)

Auto-auto-U is a common antibody in S-s+ pregnant women of African descent, that best reacts on S+s- RBCs, as they display 50% more of GPB when compared to S-s+ cells => mimicking allo-anti-S
CONCLUSIONS

• Despite the use of systematic Rh phenotypically matched RBC units and transfusion in an intra-ethnic background, the immunization rate of SCD patients in Martinique is unexpectedly high (~20 to 30%, depending on inclusion or not of the likely naturally-occurring antibodies)

• Immunization rate significantly higher in continental France (~40% in adult SCD patients)
High prevalence of red blood cell alloimmunization in sickle cell disease despite transfusion from Rh-matched minority donors

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1Division of Hematology, The Children’s Hospital of Philadelphia, Philadelphia, PA; 2Immunohematology and Genomics Laboratory, New York Blood Center, New York City, NY; and 3Department of Pathology and Laboratory Medicine, The Children’s Hospital of Philadelphia, Philadelphia, PA

Red blood cell (RBC) transfusion is a key treatment of patients with sickle cell disease (SCD) but remains complicated by RBC immunization. In the present study, we evaluated the effects of antigen matching for Rh D, C, and E, and K and transfusion from African American donors in 182 patients with SCD. Overall, 71 (58%) chronic and 9 (15%) episodically transfused patients were alloimmunized. Fifty-five (45%) chronic and 7 (12%) episodically transfused patients were Rh immunized. Of 146 antibodies identified, 91 were unexplained Rh antibodies, one-third of which were associated with laboratory evidence of delayed transfusion reactions. Fifty-six antibodies occurred in patients whose RBCs were phenotypically positive for the corresponding Rh antigen and 35 in patients whose RBCs lacked the antigen and were transfused with Rh-matched RBCs. High-resolution RH genotyping revealed variant alleles in 87% of individuals. These data describe the prevalence of Rh alloimmunization in patients with SCD transfused with phenotypic Rh-matched African American RBCs. Our results suggest that altered RH alleles in both the patients and in the donors contributed to Rh alloimmunization in this study. Whether RH genotyping of patients and minority donors will reduce Rh alloimmunization in SCD needs to be examined. (Blood. 2013;122(6):1062-1071)
CONCLUSIONS

- The rate of alloimmunization in such studies may be, however, overestimated for Rh and MNS due to the rather common "mimicking alloantibodies"

- Blood donors of Martinique show a very high degree of phenotypic similarities with SCD patients, but there likely exists a significant difference in terms of blood group allele polymorphism

- 32% of Rh antibodies => relevance for SCD patients in Martinique of a systematic RHCE allele matching? Ongoing discussions in terms of cost-efficiency approach
ACKNOWLEDGEMENTS

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