Antigen matching for patients with SCD: experience in the United States

Stella T. Chou, MD
AABB Annual Meeting
October 14, 2018
I have no conflicts of interest to disclose
Learning Objectives

• Discuss strategies to reduce alloimmunization in patients with SCD
• Discuss contribution of RH genetic variation to alloimmunization in patients with SCD
• Discuss the feasibility of RH genotype matching for patients with SCD
Alloimmunization in patients with SCD

- Remains problematic despite phenotype matching strategies
  - Anti-Rh antibodies remain most common
- Differences between donor versus recipient red cell antigen phenotypes
- Variant $RH$ alleles are common in patients with SCD and contribute to Rh alloimmunization
- Improved methods to match red cells are still needed
Strategies to mitigate alloimmunization in US

Alloimmunization prevalence

- ABO, D: 18-76%
- C, E, K: 5-27%
- + AA donors: 15-58%
- Jk^a/Jk^b, Fy^a/Fy^b, S: 7%

Rate (alloantibodies per 100 units)

- ABO, D: 1.7-3.5
- C, E, K: 0.26-0.5
- + AA donors: 0.3
- Jk^a/Jk^b, Fy^a/Fy^b, S: 0.1

Alternative strategy: transfusion from minority donors

• Blue Tie Tag Program (1995)
  – SCD Association of America
  – American Red Cross
  – Children’s Hospital of Philadelphia

• Directs blood from African-American donors to children with SCD

• Donors self-identify ethnicity

• 1500 – 1800 donations/month

Sesok-Pizzini, Immunohematology 2006
Alloimmunization despite Rh-matched RBCs from minority donors

91 unexpected Rh antibodies (>60%)

- 59 pts - episodic and 123 pts - chronic RBC transfusions
- 44,482 total exposures over 15 year period
30% antibodies were associated with laboratory evidence of DTR
(54 of 178 antibodies with specificity)

Coleman and Chou, unpublished
**RHD and RHCE genetics**

- Chr 1
- **RHD** → **RHCE**
- >500 different alleles
- >150 different alleles

**Gene Conversion**

Generates hybrid alleles and proteins
- Part of **RHD** into **RHCE**, or
- Part of **RHCE** into **RHD**

Results in partial Rh antigens with loss of Rh epitopes and/or generates new antigens

*RH* variants occur in ~1% Europeans
Much more frequent in Africans, nearly 90%
Rh variants contribute to alloimmunization

- Variant alleles may encode partial antigens, cause loss of high prevalence antigens and/or generate novel antigens
- Serologic Rh typing detects the five principal antigens and do not reliably distinguish Rh variants
RH heterogeneity in patients with SCD

RHCE

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>ce</td>
<td>0.2460</td>
</tr>
<tr>
<td>ce(48C)</td>
<td>0.1959</td>
</tr>
<tr>
<td>ce(733G)</td>
<td>0.1285</td>
</tr>
<tr>
<td>ce(48C,733G)</td>
<td>0.0729</td>
</tr>
<tr>
<td>ce(254G)</td>
<td>0.0476</td>
</tr>
<tr>
<td>ceS</td>
<td>0.0390</td>
</tr>
<tr>
<td>ceTI</td>
<td>0.0243</td>
</tr>
<tr>
<td>ceMO</td>
<td>0.0091</td>
</tr>
<tr>
<td>ceCF</td>
<td>0.0030</td>
</tr>
<tr>
<td>ceHAR</td>
<td>0.0005</td>
</tr>
<tr>
<td>ceJAL</td>
<td>0.0015</td>
</tr>
<tr>
<td>ceAR</td>
<td>0.0025</td>
</tr>
<tr>
<td>ceEK</td>
<td>0.0035</td>
</tr>
<tr>
<td>ceTI type 2</td>
<td>0.0010</td>
</tr>
<tr>
<td>ceBl</td>
<td>0.0015</td>
</tr>
<tr>
<td>ce-D(9-10)</td>
<td>0.0005</td>
</tr>
<tr>
<td>ce(733G,1006T)</td>
<td>0.0010</td>
</tr>
<tr>
<td>ce(254G,733G)</td>
<td>0.0010</td>
</tr>
<tr>
<td>ce(48C,254G,733G)</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

- > 50% RHCE altered
  - some lack high prevalence hrB and hrS
- > 30% RHD altered
- Patients with partial D, C, and e at risk for alloimmunization despite typing antigen+
- RBCs “matched” for D, C, E are not truly Rh-matched RBCs

988 patients
Inheritance of *RH* variants is just one piece of the puzzle in Rh alloimmunization.

Exposure to African American (AA) donors with Rh variants likely contributes to complex Rh alloimmunization.

Chou and Westhoff, Blood 2013
African-American (AA) donors are necessary to support transfusion therapy for SCD.

Using a Caucasian donor inventory depletes RhD- RBCs

Chou and Westhoff, Blood 2018
"Rh-matched" red cells are not truly matched

- Transfused with Rh matched (D, C, E) red cells from African American donors
- 175 antibodies among 550 transfused individuals
  - 50 occurred in antigen+ patient with at least 1 conventional allele
  - 62 occurred in antigen- patient receiving antigen- units
**RH diversity in patients with SCD vs AA donors**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Patients</th>
<th>AA Donors</th>
<th>P value</th>
<th>Gene</th>
<th>Patients</th>
<th>AA Donors</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deleted D</td>
<td>0.1289</td>
<td>0.1167</td>
<td>0.3577</td>
<td>ce conventional</td>
<td>0.2456</td>
<td>0.2726</td>
<td>0.1086</td>
</tr>
<tr>
<td>RHDψ</td>
<td>0.0292</td>
<td>0.0383</td>
<td>0.2023</td>
<td>ce(48C)</td>
<td>0.1931</td>
<td>0.1925</td>
<td>1.0</td>
</tr>
<tr>
<td>DIIIa-CE(4-7)-D</td>
<td>0.0309</td>
<td>0.0256</td>
<td>0.4288</td>
<td>ce(733G)</td>
<td>0.1324</td>
<td>0.1431</td>
<td>0.4403</td>
</tr>
<tr>
<td>RHD conventional</td>
<td>0.5449</td>
<td>0.5980</td>
<td>0.0053</td>
<td>ce(48C,733G)</td>
<td>0.0718</td>
<td>0.0273</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DAU0</td>
<td>0.1651</td>
<td>0.1414</td>
<td>0.0848</td>
<td>ce(254G)</td>
<td>0.0461</td>
<td>0.0426</td>
<td>0.7140</td>
</tr>
<tr>
<td>Weak partial D 4.0</td>
<td>0.0298</td>
<td>0.0094</td>
<td>0.0001</td>
<td>ceS</td>
<td>0.0420</td>
<td>0.0332</td>
<td>0.2385</td>
</tr>
<tr>
<td>DJa</td>
<td>0.0181</td>
<td>0.0136</td>
<td>0.3736</td>
<td>ceTl</td>
<td>0.0233</td>
<td>0.0187</td>
<td>0.4352</td>
</tr>
<tr>
<td>DAU3</td>
<td>0.0158</td>
<td>0.0213</td>
<td>0.3187</td>
<td>ceMO</td>
<td>0.0093</td>
<td>0.0145</td>
<td>0.2153</td>
</tr>
<tr>
<td>DIIIa</td>
<td>0.0140</td>
<td>0.0094</td>
<td>0.3019</td>
<td>ceCF</td>
<td>0.0035</td>
<td>0.0009</td>
<td>0.2524</td>
</tr>
<tr>
<td>DAU5</td>
<td>0.0128</td>
<td>0.0077</td>
<td>0.2031</td>
<td>ceEK</td>
<td>0.0035</td>
<td>0.0034</td>
<td>1.0</td>
</tr>
<tr>
<td>DAR</td>
<td>0.0035</td>
<td>0.0060</td>
<td>0.3999</td>
<td>ceAR</td>
<td>0.0023</td>
<td>0.0034</td>
<td>0.7226</td>
</tr>
<tr>
<td>DOL</td>
<td>0.0018</td>
<td>0.0034</td>
<td>0.4523</td>
<td>ceJAL</td>
<td>0.0018</td>
<td>0.0026</td>
<td>0.6922</td>
</tr>
<tr>
<td>DAU4</td>
<td>0.0012</td>
<td>0.0034</td>
<td>0.2316</td>
<td>ceTl type 2</td>
<td>0.0012</td>
<td>0.0009</td>
<td>1.0</td>
</tr>
<tr>
<td>DJa-3</td>
<td>0.0006</td>
<td>0</td>
<td>1.0</td>
<td>ceBl</td>
<td>0.0012</td>
<td>0.0034</td>
<td>0.2316</td>
</tr>
<tr>
<td>DFR</td>
<td>0.0006</td>
<td>0</td>
<td>1.0</td>
<td>ce(733G,1006T)</td>
<td>0.0012</td>
<td>0</td>
<td>0.5168</td>
</tr>
<tr>
<td>DWN</td>
<td>0.0006</td>
<td>0</td>
<td>1.0</td>
<td>ceHAR</td>
<td>0.0006</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>D(48C)</td>
<td>0.0006</td>
<td>0</td>
<td>1.0</td>
<td>ce(48C,254G,733G)</td>
<td>0.0006</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>D(835A)</td>
<td>0.0006</td>
<td>0</td>
<td>1.0</td>
<td>ce(254G,733G)</td>
<td>0.0006</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>RHΔψ-like</td>
<td>0.0006</td>
<td>0</td>
<td>1.0</td>
<td>Ce conventional</td>
<td>0.1190</td>
<td>0.1363</td>
<td>0.1712</td>
</tr>
<tr>
<td>D(667G,800T)</td>
<td>0.0006</td>
<td>0.0009</td>
<td>1.0</td>
<td>CeRN</td>
<td>0.0029</td>
<td>0.0017</td>
<td>0.7079</td>
</tr>
<tr>
<td>DAU6</td>
<td>0</td>
<td>0.0017</td>
<td>0.1652</td>
<td>Ce(733G)</td>
<td>0.0006</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>D-CE(3-7)-D</td>
<td>0</td>
<td>0.0017</td>
<td>0.1652</td>
<td>CeCW</td>
<td>0</td>
<td>0.0017</td>
<td>0.1652</td>
</tr>
<tr>
<td>Weak D type 40</td>
<td>0</td>
<td>0.0009</td>
<td>0.4065</td>
<td>ce conventional</td>
<td>0.0928</td>
<td>0.1005</td>
<td>0.5201</td>
</tr>
<tr>
<td>Weak D type 1</td>
<td>0</td>
<td>0.0009</td>
<td>0.4065</td>
<td>ce(48C)</td>
<td>0.0029</td>
<td>0.0009</td>
<td>0.4111</td>
</tr>
</tbody>
</table>

**Bold** = comparison of allele frequency between patients and donors with p value < 0.05 by Fisher's exact test.

587 donors
857 patients
Virtual matching with actual red cell unit demand at a single institution

~7K units transfused to 200 patients with SCD in 1 year (27 units/weekday)

<table>
<thead>
<tr>
<th>Calendar Year</th>
<th>Total units transfused</th>
<th>Patients transfused</th>
<th>Avg units issued/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013</td>
<td>7588</td>
<td>199</td>
<td>29</td>
</tr>
<tr>
<td>2014</td>
<td>7581</td>
<td>195</td>
<td>29</td>
</tr>
<tr>
<td>2015</td>
<td>6903</td>
<td>205</td>
<td>26</td>
</tr>
<tr>
<td>2016</td>
<td>6107</td>
<td>201</td>
<td>23</td>
</tr>
</tbody>
</table>
Virtual matching for genotype matched RBCs

- Donors: 10-300 donors*/day
- Day X: Donor Inventory
  - Add daily donations
  - Find matches for patient demand
  - Order filled vs not filled
  - Age inventory

- Discard units >21 days old

EUR published genotype frequencies

AA observed genotype frequencies

ABO plus matching for:
1. Serologic DCEK
2. Serologic DCEK + extended
3. RH genotype
4. RH genotype + extended

*oldest units first, reserve units with less common antigen combinations; five simulations per variable
RH genotype matching

<table>
<thead>
<tr>
<th>Patient</th>
<th>Donor</th>
<th>RH allele match</th>
</tr>
</thead>
<tbody>
<tr>
<td>RHD, ce733G, DAU0, ce48C</td>
<td></td>
<td>RHD, ce48C</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patient</th>
<th>Exact RH match</th>
<th>RH haplotype match</th>
<th>RH allele match</th>
</tr>
</thead>
<tbody>
<tr>
<td>RHD, ce733G</td>
<td>RHD, ce733G</td>
<td>DAU0, ce48C</td>
<td>RHD, ce48C</td>
</tr>
<tr>
<td>DAU0, ce48C</td>
<td>RHD, ce733G</td>
<td>DAU0, ce48C</td>
<td>DAU0, ce48C</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patient</th>
<th>Less restrictive match</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>RHD, ce733G</td>
<td>DAU0, ce48C</td>
<td>RHD, ce</td>
<td>RHD, ce</td>
</tr>
<tr>
<td>RHD, ce</td>
<td></td>
<td>RHD, ce</td>
<td>RHD, ce</td>
</tr>
</tbody>
</table>

Less restrictive match: $RHD^{*DAU0}$ and $RHCE^{*ce48C}$ as equivalent to conventional alleles
Current serologic RBC matching strategies

- Serologic ABO DCEK matching
  - 100% demand filled with ~50 AA vs ~150 Caucasian donors/day

- Extended serologic matching
  - ABO DCEK Fy Jk Ss
  - 95% demand met with ~100 AA donors/day and 43% met with 100 Caucasian donors/day

- Using Caucasian donors relies on D- donors
RH genotype matched RBCs is feasible with an African American donor pool

- 95% of patient demand met with 85 AA donors/day
- Reached maximum of 76% demand met with 150 Caucasian donors/day
- Caucasian donors rely heavily on D- donors
Difficult to match patients

• Two patients with no matches
  • \(RHCE^{*ceTI/ceTI}\) – chronically transfused (165 units)
  • \(RHCE^{*ceTI/ceEK}\) - episodic transfusion (3 units)

• Directed donor recruitment needed for patients with rare RH genotypes
  • Genotyping can identify donors with uncommon phenotypes
Summary

- RH genotyping of red cells may improve matching of patients and donors and ↓ Rh alloimmunization
- RH genotype matching may improve use of an African American donor inventory
  - ~Twice the # of donors needed for RH genotype + K matching compared to serologic Rh, K matching
  - ~25% increased donor number needed with less restrictive genetic matching - higher feasibility
- Barriers are cost of genotyping as well as inventory and data management of donor genotypes
Acknowledgements

CHOP
Perry Evans
David Friedman
Sarita Coleman
Tannoa Jackson
Devin Cohen
Kim Smith Whitley
Patients and families

NY Blood Center
Connie Westhoff
Sunitha Vege

Penn-Jersey ARC
Margaret Keller
Sandra Nance

DORIS DUKE
CHARITABLE FOUNDATION

NATIONAL BLOOD FOUNDATION

National Heart, Lung, and Blood Institute
Antigen Matching in Sickle Cell Disease Patients: Chances and Challenges in Molecular Times-The Brazilian way

Lilian Castilho, PhD
Hemocentro Campinas, Unicamp
Campinas-SP-Brazil
Disclosure

I have no relevant financial relationships to disclose for this session
Objectives

- SCD in Brazil
- RBC alloimmunization in patients with SCD in Brazil
- RH genotypes found in SCD patients from Brazil
- Brazilian experience of molecular matching: chances and challenges
Sickle Cell Disease (SCD) in Brazil

- SCD is the most prevalent hereditary disease
- ~30,000 patients nationwide
- ~4000 affected newborns each year
- ~50% are chronically transfused
- Incidence of RBC alloimmunization ranges from 10% to 60%
SCD patients treated at Unicamp Hospital

- Type of hospital: major academic adult
- Reference for more than 6 million inhabitants
- Number of beds: 615
- 368 patients with SCD homozygous HbSS
- 167 (45.4%) are currently on chronic transfusion
  - 67 (40%) patients are alloimmunized against RBC antigens
- ~20,000 leukoreduced RBC units are being transfused to the patients per year
73% of the patients have at least one antibody against Rh antigens.
SCD patients transfused at Hemocentro-Unicamp

Transfusion strategies proposed to mitigate alloimmunization in SCD

- RBCs phenotypically-matched
  - 1994-2000:
    - Rh (D, C, E, c, e) and K (patients with no RBC antibodies)
    - Rh (D, C, E, c, e), K and extended (FY, JK, MNS, DI) (patients with RBC antibodies)
  - 2000: Rh (D, C, E, c, e), K and extended (all SCD patients)
- We can most often meet the transfusion needs of the patients with SCD
- Incidence of alloimmunization decreased
- Patients still produced Rh antibodies
SCD patients transfused at Hemocentro-Unicamp

- 2002: Molecular genotyping
  - Improvement of the clinical outcomes
  - Reduction on the rates of alloimmunization
  - Patients still produced Rh antibodies

- 2008: HEA BeadChip was introduced

- 2018: All patients are genotyped with the HEA BeadChip platform

- 2010: RH variant alleles are being identified
Most prevalent *RHD* and *RHCE* alleles found in SCD

32/167 (19.2%) of patients have *RH* variant alleles

<table>
<thead>
<tr>
<th>RHD</th>
<th>%</th>
<th>RHCE</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>RHD conventional</td>
<td>69.2</td>
<td>ce conventional</td>
<td>28.1</td>
</tr>
<tr>
<td>RHD*Ψ</td>
<td>4.3</td>
<td>RHCE*ce48C</td>
<td>17.2</td>
</tr>
<tr>
<td>RHD*weak partial D 4.0</td>
<td>3.4</td>
<td>RHCE*ce733G</td>
<td>16.8</td>
</tr>
<tr>
<td>RHD*DIIIa-CE(4-7)-D</td>
<td>3.1</td>
<td>RHCE*ce48C, 733G</td>
<td>9.6</td>
</tr>
<tr>
<td>RHD*DAR</td>
<td>2.4</td>
<td>RHCE*ceS</td>
<td>3.2</td>
</tr>
<tr>
<td>RHD*DIIIa</td>
<td>2.1</td>
<td>RHCE*ceAR</td>
<td>2.8</td>
</tr>
<tr>
<td>RHD*DAU0</td>
<td>1.8</td>
<td>RHCE*ceEK</td>
<td>1.8</td>
</tr>
<tr>
<td>RHD*DIVa</td>
<td>1.6</td>
<td>RHCE*ce 733G, 1006T</td>
<td>1.6</td>
</tr>
<tr>
<td>RHD*DAU4</td>
<td>0.9</td>
<td>RHCE*ceMO</td>
<td>1.3</td>
</tr>
<tr>
<td>RHD*DOL</td>
<td>0.5</td>
<td>RHCE*ceTI</td>
<td>0.9</td>
</tr>
<tr>
<td>RHD*DAU3</td>
<td>0.5</td>
<td>RHCE*ceJAL</td>
<td>0.5</td>
</tr>
</tbody>
</table>
**RH altered genotypes and antibodies in SCD**

13/32 (40.6%) patients with Rh antibodies

<table>
<thead>
<tr>
<th>Patients (n)</th>
<th>RHD-CE genotypes</th>
<th>Rh antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RHD/RHCE*ce733G</td>
<td>Anti-e</td>
</tr>
<tr>
<td>1</td>
<td>RHD<em>DIIIa/RHCE</em>ce48C,733G</td>
<td>Anti-D, -e</td>
</tr>
<tr>
<td>1</td>
<td>RHD<em>DIIIa/RHCE</em>ce</td>
<td>Anti-D</td>
</tr>
<tr>
<td>2</td>
<td>RHD<em>DIIIa-CE(4-7)-D/RHCE</em>ceS</td>
<td>Anti-C, -hrB, -HrB</td>
</tr>
<tr>
<td>1</td>
<td>RHD<em>DIIIa-CE(4-7)-D/RHCE</em>ceS</td>
<td>Anti-hrB, -HrB</td>
</tr>
<tr>
<td>1</td>
<td>RHD/RHCE*ceEK</td>
<td>Anti-hrS</td>
</tr>
<tr>
<td>2</td>
<td>RHD/RHCE*ceAR</td>
<td>Anti-e</td>
</tr>
<tr>
<td>2</td>
<td>RHD<em>DAR/RHCE</em>ceAR</td>
<td>Anti-D, -e</td>
</tr>
<tr>
<td>1</td>
<td>RHD<em>weak partial D 4.0/RHCE</em>ce733G</td>
<td>Anti-e</td>
</tr>
<tr>
<td>1</td>
<td>RHD<em>DAU0/RHCE</em>ceMO</td>
<td>Anti-hrS</td>
</tr>
</tbody>
</table>
SCD patients transfused at Hemocentro-Unicamp

Transfusion strategies proposed to mitigate alloimmunization in SCD

2010: Three levels of molecular matching: chronically transfused patients

- **RH, KEL (level 1):** patients with no antibodies
- **RH, KEL, FY, JK, MNS, DI (level 2):** patients with antibodies
- **RH genotype matching (level 3):** patients with RH variants

- **Level 1 plus level 3:** patients with RH variants
- **Level 2 plus level 3:** patients with RH variants and antibodies

- 80% of the donor units are located using serological techniques
- Only 10% of the units are genotyped for RH variants
Compatible donors for SCD patients in 3 levels of molecular matching

Molecular Matching in patients with SCD

Total of RBC units requested and a number of 2 donations per year for the compatible donors
RBC minor antigens among the SCD patients and African Brazilian donors

<table>
<thead>
<tr>
<th>Alleles</th>
<th>Patients %</th>
<th>Donors %</th>
</tr>
</thead>
<tbody>
<tr>
<td>RHCE*C</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>RHCE*c</td>
<td>0.87</td>
<td>0.6</td>
</tr>
<tr>
<td>RHCE*E</td>
<td>0.22</td>
<td>0.03</td>
</tr>
<tr>
<td>RHCE*e</td>
<td>0.84</td>
<td>0.78</td>
</tr>
<tr>
<td>KEL*1</td>
<td>0.75</td>
<td>0.62</td>
</tr>
<tr>
<td>KEL*2</td>
<td>0.38</td>
<td>0.44</td>
</tr>
<tr>
<td>FY*A</td>
<td>0.25</td>
<td>0.2</td>
</tr>
<tr>
<td>FY*B</td>
<td>0.73</td>
<td>0.56</td>
</tr>
<tr>
<td>FY*B-67C</td>
<td>0.44</td>
<td>0.27</td>
</tr>
<tr>
<td>JK*A</td>
<td>0.38</td>
<td>0.39</td>
</tr>
<tr>
<td>JK*B</td>
<td>0.03</td>
<td>0.25</td>
</tr>
<tr>
<td>GYPB*S</td>
<td>0.97</td>
<td>0.61</td>
</tr>
<tr>
<td>GYPB*s</td>
<td>0.03</td>
<td>0.25</td>
</tr>
</tbody>
</table>
Most prevalent RH alleles found in SCD and African Brazilian donors.
Donors in a country with a mixed population

- African, Amerindian and European ancestral genomes contribute to Brazilian population’s genetic background

- European ancestral genome contributes to ~40% of the genetic ancestry of donors classified as black based on their skin color

- Genotype frequencies of the most immunogenic RBC antigens differ between donors and SCD patients (RHCE*C/c, FY*A/B, JK*A/B)

- Provide race-matched RBC units as a strategy to prevent alloimmunization is not an option
Approaches to identify RH variant donors in Brazil

- DNA samples from self-declared African Brazilian donors with the -67C allele
- DNA samples from repeated donors of group O phenotyped as C-E-K- VS+ and/or V+
- DNA samples with discrepancies in the RhD typing
- DNA samples from donors typed as D-C+ are being tested for \( RHCE*(C)ce^S \) haplotype in order to identify Hr^B- donors
Transfusion strategy to mitigate alloimmunization in non-alloimmunized SCD patients

Red blood cell alloimmunization in patients with sickle cell disease: correlation with HLA and cytokine gene polymorphisms

Emilia Angela Sipper,1 Jeane Ellete Laguita Visentainer,2 Hugo Vicentin Alves,2 Camila Rodrigues,2 Simone Cristina Olencki Gilli,1 Marcelo Addas-Carvalho,1 Sara Teresinha Oikawa Saad,1 Fernando Ferreira Costa,1 and Lilian Castilho1

**TNFA –308G/A**

**IL1B –511C/T**

**HLA–DRB1*15**

**Responder patients with SCD**

**Non-responders patients with SCD**

**Cost effectiveness**

Transfusion with level 3 of matching to responders in order to avoid production of Rh antibodies
Risk factors associated with RH alloimmunization: \textit{HLA^{*}DRB15, TNFA–308A, IL1B–511T}

- 1 patient with \textit{HLA^{*}DRB15 and IL1B–511T (RHCE^{*}ce733G/RHCE^{*}ce733G)}
- 1 patient with \textit{HLA^{*}DRB15 (RHD*DAR/RHCE^{*}ceMO)}
- 1 patient with \textit{HLA^{*}DRB15 and TNFA–308A (RHD*DAR/RHCE^{*}ceAR)}
- 1 patient with \textit{HLA^{*}DRB15 and IL1B–511T (RHD*DIIIa-CE(4-7)-D/RHCE^{*}ceS)}

- 2/19 (10.5\%) patients are being transfused with level 1 and level 3 of matching
  - 1 patient developed anti-Js\textsuperscript{a}
  - 1 patient developed anti-Fy3
- 2/19 (10.5\%) patients are being transfused with level 2 and level 3 of matching
  - 1 patient developed anti-Hr\textsuperscript{B}

- 15/19 (78.9\%) patients are not being transfused with RH genotype matching
  - No risk factors associated with RBC alloimmunization
  - No RH alloimmunization observed after two years of transfusion follow up
27 year-old female patient with SCD non-alloimmunized

- Hb: 8.3g/dL
- Phenotype: O r´r, K-, Fy(a-), Jk(b-)
- RH genotype: \( RHD^{DIIIa-CE(4-7)-D/RHCE^*(C)ceS} \)
- Being transfused with 2 RBC units each 3 weeks
- Risk factors associated with RBC aloimmunization: \( IL1B-511T \) and \( HLA^{*DRB15} \)
- Transfusion recommendation: Level 2 and level 3 of matching
- Number of units requested (2017): 36
- Number of units transfused with level 2 and level 3 of matching: 26
- Number of units transfused with level 2 of matching: 10

The number of RH genotyped donors was limited, especially for the SCD patient’s needs
Case 1
Transfusion follow up

<table>
<thead>
<tr>
<th>% HbS</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>25.8</td>
<td>23.8</td>
<td>24.3</td>
<td>25.3</td>
<td>26.1</td>
<td>45.8</td>
<td>44.3</td>
<td>29.6</td>
<td>28.8</td>
<td>29.3</td>
<td>26.1</td>
<td>24.2</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hb(g/dL)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.4</td>
<td>7.8</td>
<td>7.9</td>
<td>8.9</td>
<td>7.9</td>
<td>6.8</td>
<td>7.2</td>
<td>8.2</td>
<td>8.0</td>
<td>9.0</td>
<td>9.2</td>
<td>8.5</td>
<td></td>
</tr>
</tbody>
</table>

Anti-HrB
RH genotype matching
37 year-old female patient with SCD alloimmunized

- Hb: 8.1g/dL
- Phenotype: O R_0 r, K-, Jk(b-)
- RH genotype: RHD+/RHCE*ce48C/RHCE*ceJAL
- Antibodies developed: Anti-C, anti-e
- Being transfused with 2 RBC units each 2 weeks
- Risk factors associated with RBC aloimmunization: TNFA-308A and HLA*DRB15

Transfusion recommendation: RHD+, RHCE*ce48C/RHCE*ceJAL; K-, Jk(b-)

MMA (anti-e): < 5%
Case 2
Transfusion follow up
Antigen matching in patients with SCD

Challenges

- Heterogeneity of efficacy of alloimmunization prophylaxis protocols applied by the pediatric transfusion services
- Transfusion of non-matched RBC units in another hospital
- Presence of RH variant alleles and risk of alloimmunization
- Increased miscegeny in blood donors
  - Higher degree of admixture of African and European genomes
  - Antigenic differences in relation to SCD patients
  - Finding good approaches for screening donors with homozygous genotypes and RH variants
ACKNOWLEDGMENTS

Mayra Dorigan de Macedo
Tamires Delfino dos Santos
Sheila de Fatima P Menegati
Emília Sippert

Dr Simone Gilli
Dr Jordão Pellegrino Jr

Dr Celso Bianco
Thank you for your attention!

castilho@unicamp.br
Antigen Matching in Sickle Cell Disease Patients: Chances and Challenges in Molecular Times

The situation in France

France Pirenne, EFS Hôpital Henri Mondor, Créteil, France
france.pirenne@efs.sante.fr

American Association of Blood Bank, Boston, USA, October 12-17, 2018
Sickle cell disease in France

60% of the patients

15,000 patients

80%: Sub-Saharan African origin

French overseas territories

3,000 patients

Mayotte

Reunion

Guyane

Guadeloupe

Martinique
Transfusion in France

French Blood agency: Etablissement Français du Sang (EFS)

- Blood activities in France are under the exclusive liability of EFS: collections, screening and qualification of blood donations, preparation and distribution of blood products

- EFS is a public agency with national competences

- 10,000 employees including more than 1,000 physicians

- 13 Regional Blood Centers
  - 10 metropolitans and 3 overseas
  - 153 local sites
  - 20 research teams

3,000,000 Blood products (80% of pRBCs)/year from 1,900,000 donors
Donors with a D+C-E- phenotype

50,000 donors

3% of the donors
In the Parisian area:

5544 transfused patients are recorded in the EFS regional information system.

They received 25,000 pRBCs/year.

30.7% are immunized.
Typing strategy for blood products and patients

• **Blood products** :
  • Mandatory : typed by **serology**
    • ABO, RH (D, C, E, c, e) and K
  • Additionally, 15% of pRBCs are typed by **serology** for FY, JK, MNS, including all pRBCs from donors of African ancestry

• **Patients** :
  • Mandatory : typed by **serology**
    • ABO/RH (D, C, E, c, e) and K
    • When immunized, typed by **serology** for the specificity of the antibody
  • Additionally : SCD patients are always typed by **serology** for FY, JK, MNS
Matching strategy of SCD patients

• Non immunized patients
  • Matched for ABO, RH (D, C, E, c, e) and K

• Immunized patients
  • Only RH Abs (in negative RH antigen patients) or auto Abs or Abs against low frequency
    • Matched for ABO, RH (D, C, E, c, e) and K
  • Significant Abs (FY, JK, MNS, DO ..)
    • Matched for ABO, RH (D, C, E, c, e) and K + FY, JK, MNS (S,s)

• History of DHTR (independently of the immunization status)
  • Matched for ABO, RH (D, C, E, c, e) and K + FY, JK, MNS (S,s)
The place of molecular analysis in SCD

• Investigation of a complex screening test when a patient has been recently transfused and prior typing data not available

• Rare blood typing

• RH variants with the goal to genotype all patients, except those known as low responders

• Screening of donors
Genotyping in Sickle Cell Disease Patients: The French Strategy

Aline Floch\textsuperscript{a,b,c,d}, Christophe Tournamille\textsuperscript{a,b,c}, Btissam Chami\textsuperscript{a}, France Pirenne\textsuperscript{a,b,c,d}

\textsuperscript{a}Etablissement Français du Sang (EFS) – Île de France, Créteil, France;
\textsuperscript{b}INSERM U955, Equipe 2 ‘Transfusion et maladies du globule rouge’, Créteil, France;
\textsuperscript{c}Laboratory of Excellence GR-Ex, Créteil, France;
\textsuperscript{d}Institut Mondor de Recherche Biomédicale (IMRB), Université Paris Est-Créteil (UPEC), Faculté de Médecine, Créteil, France
French strategy for RH genotyping in France

• Based on:
  
  • Prevalence of partial RH antigens in SCD patients in our country
<table>
<thead>
<tr>
<th>Common or short name</th>
<th>ISBT allele reference*</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>haplotypes : at least partial C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RN</td>
<td>RHD<em>01 associated with RHCE</em>02.10.01</td>
<td>Le Pennec et al. Rouillac et al. Tournamille et al.</td>
</tr>
<tr>
<td>Cce$</td>
<td>RHD*03N.01 or <em>01N.06 associated with RHCE</em>01.20.03</td>
<td>Westhoff et al. Tournamille al.</td>
</tr>
<tr>
<td>DIVa(C)—</td>
<td>RHD<em>04.01 associated with RHCE</em>CE-DIVa.2(2-3)-CE-D(5)-CE</td>
<td>Hipsky et al.[21]</td>
</tr>
<tr>
<td>RHD alleles : partial D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAU alleles</td>
<td>RHD*10</td>
<td>Srivastava et al.</td>
</tr>
<tr>
<td>DAR alleles</td>
<td>RHD*09.01, .02, .06</td>
<td>Hemker et al. Wagner et al.</td>
</tr>
<tr>
<td>DIII type 5</td>
<td>RHD*03.01</td>
<td>Westhoff et al.</td>
</tr>
<tr>
<td>DIV type 4</td>
<td>RHD*04.04</td>
<td>Rouillac et al. von Zabern et al.</td>
</tr>
<tr>
<td>DV alleles</td>
<td>RHD*05</td>
<td>Rouillac et al.</td>
</tr>
<tr>
<td>weak D type 4.0 †</td>
<td>RHD*09.03</td>
<td>Wagner et al.</td>
</tr>
<tr>
<td>DOL alleles</td>
<td>RHD*12</td>
<td>Flegel et al.</td>
</tr>
<tr>
<td>RHCE*ce alleles : partial e and/or c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ceS</td>
<td>RHCE*01.20.03</td>
<td>Pham et al.</td>
</tr>
<tr>
<td>ceAG</td>
<td>RHCE*01.06.01</td>
<td>Westhoff et al.</td>
</tr>
<tr>
<td>ceMO</td>
<td>RHCE*01.07.01</td>
<td>Noizat-Pirenne et al. Westhoff.</td>
</tr>
<tr>
<td>ceAR</td>
<td>RHCE*01.04.01</td>
<td>Noizat-Pirenne et al. Peyrard et al. Hipsky et al.</td>
</tr>
<tr>
<td>ceEK</td>
<td>RHCE*01.05.01</td>
<td>Noizat-Pirenne et al. Peyrard et al.</td>
</tr>
<tr>
<td>ceBI</td>
<td>RHCE*01.08</td>
<td>Reid et al.</td>
</tr>
<tr>
<td>ceSM</td>
<td>RHCE*01.09</td>
<td>Reid et al.</td>
</tr>
<tr>
<td>ceJAL</td>
<td>RHCE*01.20.07</td>
<td>Lomas-Francis et al. Ong et al.</td>
</tr>
<tr>
<td>ceTI type alleles</td>
<td>RHCE*01.02, *01.03, *01.20.04</td>
<td>Westhoff et al.</td>
</tr>
<tr>
<td>ceCF ‡</td>
<td>RHCE*01.20.06</td>
<td>Hipsky et al.</td>
</tr>
</tbody>
</table>
French strategy for RH genotyping in France

• Based on:

  • Prevalence of partial RH antigens in SCD patients in our country

  • Clinical relevance of partial RH variant in SCD

  • However, we need to distinguish high and low responder patients to alloimmunization
    • No need to type RH variants in heavily transfused low responders patients ...

  • TYPING ONLY FOR NEW PATIENTS AND IMMUNIZED PATIENTS
Molecular work up in SCD takes part in the effort to optimize transfusion safety in SCD

1 case of a patient who developed DHTR due to anti-RH46 antibody

The Rare RH:-46 blood group was not known
Organization of the molecular immunohematology laboratories

4 LABORATORIES

54%
Creteil LIHM
samples from the Parisian area, Guadeloupe, French Guiana, and other mainland French regions

65% of SCD patients
In France

25%
Marseille LIHM
South of France, Réunion Island, Mayotte

CNRS laboratory:
additional analyses
for complex immunohematological cases

21%
Brest LIHM
West of France
Implementation steps of RH genotyping

• Since 2010 in the Parisian area (Laboratory of Creteil)
  • All RhC+ SCD patients are genotyped for RHC partial variants with or without ambiguous C phenotype
  • Based on a study showing high prevalence of partial RhC with anti-C (Tournamille, Transfusion, 2010)

Cc genotyping

C (RH2) polymorphisms detected
(C/C and C/c)

RN haplotype detection

if not present: considered normal C

Detection of Cces haplotypes

if neither is detected: patient is considered “uncharacterized partial C”

only c (RH4) polymorphisms
(c/c)

DIVa(C)– haplotype detection
Results of the prevalence of RhC partial antigens: samples tested from 2010 to 2017

<table>
<thead>
<tr>
<th>RHCE allele</th>
<th>Parisian area</th>
<th>Guadeloupe</th>
<th>French Guiana</th>
<th>All regions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of RH:2 patients tested</td>
<td>701</td>
<td>176</td>
<td>61</td>
<td>963</td>
</tr>
<tr>
<td>RN haplotype</td>
<td>83 (11.8%)</td>
<td>2 (1.1%)</td>
<td>1 (1.6%)</td>
<td>90 (9.3%)</td>
</tr>
<tr>
<td>Cce^S haplotypes</td>
<td>151 (21.5%)</td>
<td>33 (18.8%)</td>
<td>7 (11.5%)</td>
<td>195 (20.2%)</td>
</tr>
</tbody>
</table>
Gradual implementation of full RH analysis for SCD patients

For each SCD patient, depending on RH phenotype

For RH:1 phenotype
- BioArray RHD BeadChip®
- Non exhaustive list of the main alleles of interest (detectable according to the manufacturer):
  - *DAR and weak D types (RHD*09), DOL (RHD*12), DIVa2 (RHD*04.01), some DAU types (RHD*10), DIII type 5 (RHD*03.01), DIV type 4 (RHD*04.04), DV types (RHD*05)*

RHCE analyses
- Additional variant alleles detected by other methods:
  - *Other DAU types (RHD*10) *
- Additional variant alleles detected by other methods:
  - Heterozygous CeRN (RHCE*02.10.02), ceAG (RHCE*01.06.01)...
- BioArray RHCE BeadChip®
- Non exhaustive list of the main alleles of interest (detectable according to the manufacturer):
  - *ce alleles (RHCE*01): ce(48) (*01.01), ceTI (*01.02), ceMO (*01.07.01), ceAR (*01.04.01), and ce(733), ce(48,733), ce², ceCF, ceJAL (respectively *01.20.01 to .03, .06 and.07)*
Results of the exploration of RH and RHCE in 1,148 patients from 2014 to 2017

<table>
<thead>
<tr>
<th></th>
<th>Parisian area</th>
<th>Guadeloupe</th>
<th>French Guiana</th>
<th>Other mainland regions</th>
<th>All regions</th>
</tr>
</thead>
<tbody>
<tr>
<td>patients with ≥ 1 partial RH antigen / number of patients (%)</td>
<td>123 / 466</td>
<td>69 / 394</td>
<td>41 / 221</td>
<td>20 / 70</td>
<td>253 / 1148</td>
</tr>
<tr>
<td></td>
<td>26.5%</td>
<td>17.5%</td>
<td>18.6%</td>
<td>29.4%</td>
<td>22.0%</td>
</tr>
<tr>
<td>number of partial RH1 / all RH:1 patients (%)</td>
<td>47 / 450</td>
<td>46 / 375</td>
<td>32 / 213</td>
<td>6 / 66</td>
<td>131 / 1104</td>
</tr>
<tr>
<td></td>
<td>10.4%</td>
<td>12.3%</td>
<td>15.0%</td>
<td>9.1%</td>
<td>11.9%</td>
</tr>
<tr>
<td>number of partial RH2 / all RH:2 patients (%)</td>
<td>70 / 185</td>
<td>21 / 126</td>
<td>8 / 60</td>
<td>8 / 19</td>
<td>107 / 390</td>
</tr>
<tr>
<td></td>
<td>37.8%</td>
<td>16.7%</td>
<td>13.3%</td>
<td>42.1%</td>
<td>27.4%</td>
</tr>
<tr>
<td>number of RH:3 patients</td>
<td>77</td>
<td>86</td>
<td>36</td>
<td>9</td>
<td>208</td>
</tr>
<tr>
<td></td>
<td>1.6%</td>
<td>0.5%</td>
<td>1.4%</td>
<td>4.4%</td>
<td>1.3%</td>
</tr>
<tr>
<td>number of partial RH5 / all RH:5 patients (%)</td>
<td>26 / 462</td>
<td>25 / 392</td>
<td>13 / 220</td>
<td>7 / 68</td>
<td>71 / 1141</td>
</tr>
<tr>
<td></td>
<td>5.6%</td>
<td>6.4%</td>
<td>5.9%</td>
<td>10.3%</td>
<td>6.2%</td>
</tr>
</tbody>
</table>
Extended genotyping in SCD patients from 2015 to 2017

Rarely necessary in France because extended phenotyping is included in the initial serological workup

Patients typed for FY, JK, MNS or DO each year relative to all SCD samples genotyped
Challenges in donors of same ethnic background

• Screening for rare blood

• Screening for low frequency antigens (RH20 ..)
  • In order to match for patients who developed the antibody in history
  • The antibody against low frequency are frequently evanescent and they are not detected at the pre transfusion screening test as well as at the pre transfusion serological cross matched (Floch, Transfusion, 2018)

Implementation in discussion .....

Conclusion

• In France, molecular work up is mainly implemented for RH, however, clinical relevance needs to be investigated.
  • Variants proned to allo immunization in recipients?
  • Clinical relevance of the associated antibodies?
  • What about new variants discovered based on ambiguous antigen: partial status?

• Combining serology and molecular analysis have achieved the goal of having a good knowledge of the geno/phenotype of patients

• The main challenge is in donors
Special Thanks to

Aline Floch and Christophe Tournamille,
from the Molecular Laboratory in Créteil

Sophie Viret, Pharm Student
SN4-32 Antigen Matching in Sickle Cell Disease Patients: Chances and Challenges in Molecular Times

Sunday, October 14, 2018
02:00 PM - 03:30 PM

Moderator: Connie M. Westhoff, SBB, PhD
Executive Scientific Director
Immunohematology and Genomics, New York Blood Center
and
National Center for Blood Group Genomics, Kansas City
FACULTY DISCLOSURES

• No relevant financial interests
OBJECTIVES

• Assess susceptibility to RBC alloimmunization in patients with SCD

• Discuss efforts to mitigate risk of alloimmunization

• Evaluate the challenges for molecular typing (genotyping) of patients and donors

• Compare practices in Brazil, France, and United States
FACULTY

• **Lilian Castilho, PhD**
  – Professor and researcher at University of Campinas, Brazil
  – Scientific consultant at Albert Einstein Hospital, São Paulo, Brazil
  – Regional Director ISBT for South America

• **France Pirenne, MD**
  – Professor of Hematology and Transfusion, University Paris Est Creteil
  – Medical Director of the French Blood Agency, Paris

• **Stella T. Chou, MD**
  – Associate Professor of Pediatrics, Children's Hospital of Philadelphia