Molecular Immunohematology
User Group Meeting
April 25, 2017

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Laboratory Professionals
GET RESULTS
Medical Laboratory Professionals Week
APRIL 23-29, 2017 = LABWEEK

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Continuing Education

• Each attendee must register to receive CE at:
  https://www.surveymonkey.com/r/April2017_MIH_UserGroupWebinar

• Registration deadline is May 12, 2017

• Certificates will be sent via email only to those who have registered by May 26, 2017

Presentation Recording

• Session will be recorded and posted.
  – Access information will be sent to each registrant when the recording becomes available

• No CE issued for participating in recording

Other

• You are all muted

• Q&A following session
  – Type in questions
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Clinical benefits of blood group genotyping in patients with Sickle Cell Disease and Thalassemia

MIH User Group Meeting
April, 25th, 2017

Lilian Castilho, PhD

Objectives
• Brazilian experience and clinical benefits of blood group genotyping in patients with SCD and thalassemia
• Molecular matching
• RH genotyping in patients with SCD and donors
Sickle Cell Disease (SCD) in Brazil

- SCD is the most prevalent hereditary disease in Brazil
- 25,000-30,000 patients
- ~4000 affected newborns each year
- ~50% are chronically transfused
- 378 patients with SCD homozygous HbS in our institution
- 167 (44.2%) are currently on chronic transfusion
- ~10,000 leukoreduced RBC units are being transfused to the patients per year
- Patients are being genotyped with the HEA, RHD and RHCE BeadChip platforms
- Institutional policy for transfusion has been to antigen-match prospectively for Rh, K, Fy(a), Fy(b), Jk(a), Jk(b), S and Dr

RBC alloimmunization profile

- 167 patients
- 67 alloimmunized (40%)
- 73% of the patients have at least one antibody against Rh antigens
- 67% of the patients: outside transfusions
- Advanced age
- RH variants
- 32 (19.2%): RH variant alleles
- 13 (40%): Rh antibodies

Utility of red cell genotyping in patients with SCD

- 2002: Red cell genotyping could be an excellent tool to improve transfusion therapy for patients with SCD
- allowing the determination of the true blood group genotype, by assisting in the identification of suspected alloantibodies
- helping in decrease the risk of transfusion reactions, especially delayed transfusion reactions to existing alloantibodies, and in preventing alloimmunization
- Genotype differed from assumed phenotype in 6 of 40 transfused patients with SCD

- All patients benefited from receiving antigen-matched RBCs based on genotype, as assessed by better in vivo RBC survival, increased Hb levels, and diminished frequency of transfusion
Red cell genotyping in chronically transfused patients with SCD

Patient and donor units HEA genotyped

- Over the past fifteen years, we have demonstrated:
  - Red cell genotyping is useful to increase the availability of blood units to SCD patients with Fy(b-) phenotypes
  - Red cell genotyping is useful in preventing alloimmunization and in providing appropriate antigen-matched products

Genotyped units: 190-22 antigens
80/90 patients: minimum of 5 negative antigens

SCD Patients: n=90
RH, KEL, Fy, K, GYPB
GATA+, Fy(a+): 39%

Benefits of red cell genotyping in patients with SCD

DNA array analysis for red blood cell antigens facilitates the transfusion support with antigen-matched blood in patients with sickle cell disease

Our results demonstrated that the DNA array technology could identify blood group genotypes for SCD patients and was useful to readily identify compatible donors for them

Molecular matching in patients with SCD

Clinical benefits

- Reduction on the rates of alloimmunization
  - 5-10%: C E K matching
  - ≤ 1%: extended matching

- Improvement of the clinical outcomes
  - Hb levels and % HbS:
  - Better in vivo RBC survival
  - Diminished frequency of transfusions

<table>
<thead>
<tr>
<th>Hemoglobin S (%)</th>
<th>Hemoglobin (gm/dL)</th>
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<tbody>
<tr>
<td>Pre-transfusion</td>
<td>Post-transfusion</td>
</tr>
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<td>Post-transfusion</td>
</tr>
<tr>
<td>45.8</td>
<td>23.8</td>
</tr>
<tr>
<td>42.2</td>
<td>29.9</td>
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<tr>
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<td>21.5</td>
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<td>46.5</td>
<td>28.2</td>
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<tr>
<td>46.6</td>
<td>18.9</td>
</tr>
<tr>
<td>41.9</td>
<td>7.2</td>
</tr>
<tr>
<td>43.6</td>
<td>8.4</td>
</tr>
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</table>
Molecular matching in patients with SCD

Compatible donors for SCD patients in 3 levels of molecular matching

Total of RBC units requested and a number of 2 donations per year for the compatible donors

Clinical benefits of red cell genotyping in SCD patients

<table>
<thead>
<tr>
<th>Alleles</th>
<th>Predicted phenotypes</th>
<th>Antibodies</th>
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<tbody>
<tr>
<td>RHCE*ce(48C)</td>
<td></td>
<td>Anti-e</td>
</tr>
<tr>
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<td>Anti-e</td>
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RH variant alleles and clinically significant Rh antibodies in SCD patients

Patient: JOS, male, 35 years
Phenotype: O D+C-E+ (R+y)
Identified antibody: anti-D
RH variant: RH*D*DAU4
Receiving D-E-C-E+ (y) RBC units
- After transfusion of 12 RBC units: anti-e
- RHCE variant: RHCE*ce(733G)
Clinical benefits of RH genotype matching in SCD patients

Patient: SAO, male, 28 years
Phenotype: O D-C-c-e+/e [r]
Identified antibody: anti-C
RH variants: RHCE*(C)ce/S / RHCE*(C)ceS

Clinical benefits of RH genotype matching in SCD patients

Patient: MJS, female, 29 years
Phenotype: O D+C-e-/E+c+e [r]
Antibody: anti-C
RH variants: RHCE*eu/RHCE*ceEK

When would RHCE allele matching be considered?

<table>
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<tr>
<th>SCD patients with clinically significant Rh antibodies</th>
<th>SCD patients with hrB-, hrS- phenotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>15%</td>
<td>7%</td>
</tr>
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</table>

RHD and RHCE alleles

Specific RHCE allele
Molecular matching in patients with SCD

Challenges

- Transfusion of non-matched RBC units in another hospital
- Presence of RH variants
  - ~ 19% of patients
- Only 5% of RBC units are genotyped for RH variants
- Increased miscengenation in blood donors classified as Blacks
- Higher degree of admixture of African and European genomes
- In some patients, more closely matched donors are necessary
- Cost and reimbursement!

SCD patients with RH variants and alloantibodies

6/13 (46%) are receiving RH matching RBC units

<table>
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<tr>
<th>n</th>
<th>RH variant alleles</th>
<th>Predicted phenotypes</th>
<th>Antibodies</th>
</tr>
</thead>
</table>
| 1 | RHD*DAU0,RHCE*CEA | partial D, partial e | Anti-C | 4/25/2017
| 2 | RHD*DAR,RHCE*CEA | partial D, partial e | Anti-D | 4/25/2017
| 3 | RHD*DAU4,RHCE*CEA | partial D, partial e | Anti-D | 4/25/2017
| 4 | RHD*DAU0,RHCE*CEA | partial D, partial e | Anti-D | 4/25/2017

7/13 (54%) are receiving extended molecular matching but not receiving RH matching RBC units

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<th>Predicted phenotypes</th>
<th>Antibodies</th>
</tr>
</thead>
</table>
| 1 | RHD*weak D type B*4.0,RHCE*CEA | partial D, partial e | Anti-D | 4/25/2017
| 2 | RHD*DIIIa,RHCE*CEA | partial D, partial e | Anti-D | 4/25/2017
| 3 | RHD*DAR,RHCE*CEA | partial D, partial e | Anti-D | 4/25/2017
| 4 | RHD*DAU0,RHCE*CEA | partial D, partial e | Anti-D | 4/25/2017

- Prophylaxis of maintaining higher Hb levels in those patients is restricted
- Number of transfusions in such patients is lower
- Evidence of clinical risk in such patients: incompatible blood is being transfused
Future plans

- Look for more clinical and laboratory evidences
- Not all patients with Rh variants develop antibodies
- Not all Rh antibodies developed by patients with RH variants are clinically significant
- Select responders and non-responders
- Find good approaches to screen more donors with homozygous genotypes and RH variants
- Have enough donor units with RH variants to fulfill all the transfusion requests

Thalassemia in Brazil

- Brazil Thalassemia
  - ~ 3 million thalassemia minor
  - 50% thalassemia major or intermedia
- Northern region
  - Thalassemia Major: 35, 11.59%
  - Thalassemia Intermedia: 101, 50.16%
- Northeast region
  - Thalassemia Major: 22, 7.28%
  - Thalassemia Intermedia: 7, 1.78%
- Midwest region
  - Thalassemia Major: 1, 0.04%
  - Thalassemia Intermedia: 1, 0.04%
- Southeast region
  - Thalassemia Major: 65, 12.56%
  - Thalassemia Intermedia: 19, 9.41%
- South region
  - Thalassemia Major: 7, 3.47%
  - Thalassemia Intermedia: 5, 2.47%

Thalassemia in Brazil

- 65 patients in our institution
- 38% alloimmunized

Red cell genotyping in transfused patients with thalassemia

- 46% patients with discrepancies between phenotype and genotype who had changed their transfusion recommendation
- Although the blood availability has decreased for some patients, the risk of alloimmunization and HTR were reduced
Clinical Case

Polytransfused thalassemic patient, 25 years with recent transfusion (10 days)

Order for 2 units of RBC units

Phenotype: A D+C+E+c+e- (R2R2) K+, Fy(a-b+), Jk(a-b+), S+s+

Previously identified antibodies: anti-K, -Jk

Incompatible crossmatching with e-, K- Jk(a-) RBCs

Autoantibody was suspected!!

Clinical Case

Family requested

Mother’s phenotype: A D+C+E-c+e+ (R1r) ???

Patient’s phenotyping was repeated in gel with MoAbs: A R2R2?

What to do?

Exclusion of maternity????

Patient genotyping !!!!!!

Clinical Case

Predicted phenotype (HEA BeadChip)

Patient phenotype: R1r !!!!!!

Autoantibody was anti-E

Patient was transfused with A R1r K-, Fy(a-b+), Jk(a-b+), S+s+

Better in vivo RBC survival rates

Increasing the availability of blood units
Those studies provided evidence that molecular typing is superior to serological typing in chronically transfused patients. The use of better-matched blood units can reduce transfusion requirements, reducing the risk of other adverse reactions like transfusion-related acute lung injury and potential exposure to infectious disease. All patients from Brazil with thalassemia are being genotyped by HEA BeadChip.

Clinical benefits of red cell genotyping in patients with SCD and thalassemia

Molecular matching in patients with Thalassemia

Compatible donors for SCD patients in 3 levels of molecular matching

Questions?

- Is there a large enough patient population that needs extended matched RBCs?
- Can sufficient donors be typed to meet this need?
  - Traditional serologic phenotype
  - Genotyping
- How can a genotyped/phenotyped inventory be managed?
- What are the future directions of donor genotyping?
Thank you for your attention!

castilho@unicamp.br

Immucor
Molecular Immunohematology (MIH) User Group Meeting

HgbS screening of blood donors

Connie M. Westhoff, SBB, PhD
Executive Scientific Director
Immunohematology and Genomics
New York Blood Center and National Center for Blood Group Genomics

Hemoglobin S (HbS) mutation

- Nucleotide position
  - 20 A>T change; Glutamic Acid (Glu) → Valine (Val) in globin
  - HbA:HEA designated 173A>T
- **Sickle Cell Anemia/Disease (SCA/SCD)** – inherit 2 mutated genes homozygous for mutation
  - HbS/S - have chronic anemia - don't present for donation
- **Sickle Cell Trait (SCT)**, 1 mutated /1 normal gene heterozygous for mutation
  - HbS/A “carriers” - healthy and asymptomatic

Reference SNP (refSNP): rs334
Hemoglobin S screening of Blood Donors

- **HgbS/A – Sickle Cell Trait (SCT) or “Carriers”**
  - healthy, asymptomatic
  - not excluded from blood donation
  - normal hgb values

- **Units meet qualifications for routine transfusion**
  - no significant difference in ATP, 2,3-DPG, K+, glucose, lactic acid, pH, osmotic fragility
  - no sickling observed on storage


Why Hemoglobin S Testing of Blood Donors?

- **HgbS negative donor units requested:**

  1. For Patients with SCD
     - accurate monitoring of HgbS/A ratio post-transfusion
     - goal: HgbS level <30%
     - avoid interference of donor HgbS
  2. For Patients with Thalassemia
     - accurate monitoring of levels of HgbA post-transfusion
  3. Avoid possibility of sickling under low oxygen tension
     - for neonatal intensive care and newborns
     - for intrauterine transfusions (IUT’s)

Transfus Clin Biol. 2006, 3:225-33 Assessment of qualitative functional parameters of stored red blood cells from donors with sickle cell trait (AS) or with heterozygote (AC) status. Ould Amar AK et al.

Why Hemoglobin S Testing of Blood Donors?

- **100% leukoreduced blood products**
  - Problem: leukoreduction failures
  - failure to remove WBCs (less than 1 to 5 X 10^6 required)
  - associated with sickle trait HgbS/A units
  - prolonged or incomplete filtration

Leukoreduction of sickle cell trait blood: an unresolved issue. Kratides P et al.

Transfus Med Rev. 2004, 18:168-76
Leukoreduction filtration of blood with sickle cell trait. Schwartz AK et al.

Notify and defer donors from RBC donation
Hgbs Testing by New York Blood Center

- New York City: 36% foreign born
  - >12,000 patients with SCD (250 newborns/year)

<table>
<thead>
<tr>
<th>Population</th>
<th>Donors (2015)</th>
</tr>
</thead>
<tbody>
<tr>
<td>35% White</td>
<td>58% White</td>
</tr>
<tr>
<td>28% Black</td>
<td>8% Black</td>
</tr>
<tr>
<td>27% Hispanic/Latino</td>
<td>13.5% Hispanic/Latino</td>
</tr>
<tr>
<td>10% Asian</td>
<td>6.5% Asian</td>
</tr>
</tbody>
</table>

- Sickle trait (Hgbs/S) occurs in 1 in 12 minority donors: ~8%
- NYBC screening of donors:
  - all who self-identify as Black/Hispanic/Latino/Mixed
  - O negative donors to support neonatal intensive care
  - C-E-K donors who support patients with SCD
  - provide information on the unit

Hgbs Screening by Solubility

SickleScreen kit for Hgbs screening

Cloudy / turbidity = positive test
Cannot see through solution

Based on differential solubility
- reduce Hgbs is insoluble

NYBC: Screen ~200 donors/day

Screening for Hgbs by HEA

<table>
<thead>
<tr>
<th></th>
<th>HgbS</th>
<th>HgbA</th>
<th>HgbC</th>
</tr>
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<td></td>
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</tr>
<tr>
<td>HgbC</td>
<td></td>
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<td></td>
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</tbody>
</table>

0 = Hgbs−
+ = sickle trait (Hgbs/S)
++ = Hgbs/S patients with SCD
HgbS - Validation and Parallel Testing

- **Validation**
  - 4 samples discordant between SickleScreen and HEA
  - Gene sequencing performed
  - Confirmed HEA results
  - 4 samples were “false positive” SickleScreen

- **Parallel Testing**
  - 44 samples discordant between SickleScreen and HEA
  - Gene sequencing performed
  - Confirmed HEA results

<table>
<thead>
<tr>
<th>Gene Sequencing</th>
<th>HEA</th>
<th>SickleScreen</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/T</td>
<td>46</td>
<td>43</td>
</tr>
<tr>
<td>A/A</td>
<td>12</td>
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HEA BeadChip – Used for Confirmatory Test for Donors Self Identify as White

NYBC Three Years Experience 2012-14

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Number of Donors</th>
<th>Proportion of Donors</th>
<th>Positive/Negative</th>
<th>Donor Screening Results</th>
</tr>
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<tr>
<td>Asian</td>
<td>33,883</td>
<td>6.4%</td>
<td>6,230</td>
<td>110/172</td>
</tr>
<tr>
<td>Black</td>
<td>40,661</td>
<td>7.7%</td>
<td>25,251</td>
<td>1,100/20,151</td>
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<td>Hispanic</td>
<td>72,173</td>
<td>13.3%</td>
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<td>0.6%</td>
<td>1,509</td>
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<td>Total</td>
<td>122,676</td>
<td>22.3%</td>
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Donor Identification

- 48/60 negative; only 12 positive
- HPLC concordant with HEA
- Only 12 true positive
- No other atypical hemoglobin identified

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<th>SickleScreen</th>
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Summary

- **HgbS testing on donors** is a **screening test**
- **Not meant for clinical diagnosis**
- **Not mandated test**
  - In U.S. FDA recommends notifying donor
  - HEA more accurate
  - Avoids unnecessary donor notification
    - are deferred to platelet donation to maintain 100% leukoreduced RBC inventory and avoid "filtration failures"
- **HEA is superior to solubility screening**
  - No subjective reading and recording
  - Semi-automated
  - Higher-throughput

Summary

- Is this testing that requires donor consent?
  - **Considerations**
    - **Intended use** does not differ from the solubility test
    - to "screen" donors
      - we inform donors of positive results
      - counsel to follow up to confirm with their physician
    - **Recent BPAC recommendations on Donor Notification**
      - that blood donors be advised that their donations may be tested
      - that they will be notified of positive results
      - has been suggestion by some that they be able to opt out of notification
    - **FDA may eventually require centers to inform donors they may be tested**
      - Sample notification: “Donors may be tested by health related screening tests and the results will be provided directly to the donor”
Thank You!

New York Blood Center
Immunohematology and Genomics Laboratory

Protecting Access to Medicare Act and HEA Coding Changes

Kevin Trainor
Worldwide Marketing Manager, Molecular and Software

Continuing Education

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