Comparison and Validation of Chimerism in Pediatric Recipients of Hematopoietic Stem Cell Transplantation (HSCT). Comparison of quantitative Real-Time PCR (qRT-PCR) vs. Variable Number Tandem Repeats (VNTRs)

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Introduction

- Chimerism evaluation post-transplant is important to monitor engraftment, primary or secondary graft failure and potentially diagnosed early relapse.

- Several techniques have been used to monitor chimerism, such as HLA typing, STRs, VNTRs, FISH and most recently qRT-PCR.

- The most important issues regarding chimerism are:
  - to be reproducible, specific, sensitive, cost effective and with a short turn around time
Purpose and Objectives

- The purpose of the study was to compare and validate two methods to assess chimerism in pediatric patients who have undergone allogeneic hematopoietic transplantation.

- To assess the sensitivity and specificity of two methods

- To validate the newly introduced method by qRT-PCR vs. our standard of VNTRs
Materials and Methods

- Forty five samples (Peripheral Blood n=36 Bone Marrow n=9) from children who underwent HSCT for malignant or non-malignant diseases were simultaneously tested for VNTRs and AlleleSEQR (qRT-PCR).

- Samples were separated in 3 sub-sets Total WBC using the Erythrocyte Lysis Buffer (Qiagen), T cell and Myeloid using the RosetteSep™ with T cell and Myeloid enrichment kits (Stem Cell Technologies)

- DNA was extracted from each sub-set using QIAamp DNA Blood Mini kit (Qiagen)

- VNTRs were performed using APO-B, D1S80, D1S111, D17S30 alleles and gene loci SRY and ZP3

- qRT-PCR was performed using Applied Byosystems 7500R using 34 assays AlleleSEQR® (Celera) which were designed to be bi-allelic insertion/deletion polymorphism in the human genome
Statistical Analysis

- Column statistics to obtain a mean, median, ± SEM and range as well as an paired t test to compare the two methods. A p value <0.05 was determined to be significant.

- Regression analysis was performed for each sub-set comparing the two methods. An r² value close to 1 was considered optimal. PRISM software (Graph pad).

- For external validation we compared the results of the reference lab (CH) with those obtained at our lab (CMH) and performed the same analysis as outlined above (t test and regression analysis).
# Results

## Internal validation

<table>
<thead>
<tr>
<th></th>
<th>VNTR (%)</th>
<th>qRT-PCR (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ±SEM</td>
<td>92.8 ± 1.8</td>
<td>91.7 ± 2.2</td>
<td>0.10</td>
</tr>
<tr>
<td>Median</td>
<td>99</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>(42-99)</td>
<td>(35-100)</td>
<td></td>
</tr>
<tr>
<td><strong>T Cell</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ±SEM</td>
<td>94.2 ± 2.1</td>
<td>93.7 ± 2.3</td>
<td>0.22</td>
</tr>
<tr>
<td>Median</td>
<td>99</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>(37-100)</td>
<td>(29-100)</td>
<td></td>
</tr>
<tr>
<td><strong>Myeloid</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ±SEM</td>
<td>89.4 ± 3.5</td>
<td>89.6 ± 3.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Median</td>
<td>99</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>(12-99)</td>
<td>(9.3-100)</td>
<td></td>
</tr>
</tbody>
</table>
Results Internal validation

\[ r^2 = 0.94 \]
\[ p < 0.0001 \]

\[ r^2 = 0.98 \]
\[ p < 0.0001 \]
# External Validation

## Comparison of samples from CH vs. CMH

<table>
<thead>
<tr>
<th></th>
<th>CH Donor</th>
<th>CMH Donor</th>
<th>CH recipient</th>
<th>CMH recipient</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Mean ± SEM</td>
<td>59.3 ± 11.3</td>
<td>65.1 ± 10.8</td>
<td>34.6 ± 10.8</td>
<td>41.0 ± 11.3</td>
<td>Donor 0.3</td>
</tr>
<tr>
<td>Med range</td>
<td>57.6 (0-99.9)</td>
<td>73.2 (0-100)</td>
<td>25.2 (0-100)</td>
<td>42.1 (0-100)</td>
<td>Recipient 0.3</td>
</tr>
</tbody>
</table>
External Validation
Comparison of samples from CH vs. CMH

Donor Results

Recipient Results

$r^2 0.701$

$p<0.0025$

$r^2 0.707$

$p< 0.0023$
Conclusions

- The AlleleSEQR real time PCR can be used to detect chimerism.
- This method is simple and yields rapid results.
- Has the potential of providing a larger number of informative alleles.
- Correlates very well with our standard method of VNTRs.
- We have completed an internal and external validation of the test.
Conclusions

- The internal validation show no statistical difference between the two methods.

- The external validation/proficiency show some difference which could be explained by the small number of samples.

- This method is more sensitive and specific.

- This method is less time consuming with the potential of a faster turn around time.

- The cost comparison was not performed in this analysis.
Aknowledgments

From the Chimerism Lab at Children’s Memorial Hospital
- Sana Khan
- Marie Olszewski
- Wei Huang

From the City of Hope HLA Laboratory
- Dr. David Sinitzer