BACTERIAL STERILITY DATA OF APHERESIS PLATELETS COLLECTED BETWEEN 2011 AND 2016

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Background

• Bacterial infection due to contamination of platelet products is the major infectious post transfusion risk
  – static at a rate of approximately 1/1000 to 1/3000 for the last 30 years.
  – of which 1/10 will result in patient symptoms and a fatality rate of 1/100 000
  – Mortality occur around day 4 and 5 of stored platelets.
  – Globally, bacterial sepsis is underreported and definitely true for South Africa

• Potentially any bacterium can give rise to infection and or sepsis:
  – pathogenic organisms; esp. Gram negative bacteria (*E.coli, Enterobacter spp, Serratia spp.*) and Gram positive cocci (*Staph aureus*) have the highest risk for a fatal outcome.

Transfusion associated sepsis (TAS)

• TAS is the 3rd most common cause of Transfusion related deaths: 10%
• 70% PL, 30% RBC
• Causal relationship difficult to prove
  • Recipient factors: Immunosuppression
  • mortality increased for other reasons
• Diagnostic challenges : antibiotics
• 50% drop of TR mortality 2004:
  – bacterial screening
  – switching from multiple donor pooled Platelets to AP

SANBS CONTEXT

• Sterility testing can only be done at Johannesburg Head office
• Significant logistic processes to get AP samples from all provinces to Sterility testing lab
• Chronic shortage of platelets and cannot test 100%
• Mostly unable to get the minimum recommended volume
• No confirmatory testing possible (rates may be lower than presented here)
• No data on Pooled platelets
Why are platelets such high risk products?

- Platelet “media” is conducive for bacterial growth under storage conditions at 20 to 24 °C
- Aerobic condition in the absence of bactericidal factors
- Collection: 5 – 62 CFU/bag = Bacterial load < 1 CFU/ml
- Spiking studies: 1 CFU / bag results in Rapid growth : 3 days > 10⁵ CFU
- Pyrogenic substances (endotoxin)

**Bacterial Culture**: Sensitivity is volume dependent
- an early culture sampling volume of 4 mL, 8 mL, and 16 mL would respectively detect 46 percent, 71 percent, and 91 percent of bacterially contaminated platelet products at a contamination level of 0.154 CFU/mL

➢ The AABB recommends that blood services have methods in place to detect microbial contamination of all platelets

Bacterial risk control strategies from AABB, FDA to enhance the safety of platelets for transfusion

• AABB:
The AABB recommend that apheresis platelets (AP) be stored for 24 hours prior to sampling for sterility testing. After that time 4 – 8ml of the platelet component are drawn and inoculated into either one aerobic bottle or both aerobic and anaerobic bottles. The product can be released for transfusion if negative after 24 hours incubation, the culture continued for the shelf life of the unit which is usually 5 days.

• FDA (non-binding – guidance for Industry) – additional guidelines
  + Approved the use of Rapid tests for pre-release testing of platelets
  + Recommend the use of PI for apheresis platelets

AABB Technical Manual 18th Ed.

March 2016: FDA: Bacterial Risk Control Strategies for Blood Collection Establishments and Transfusion Services to Enhance the Safety and Availability of Platelets for Transfusion Draft Guidance for Industry
Aim and Methods

• To present the annual trends in terms of AP tested for bacterial contamination
  – in terms of numbers tested; percentage sterility, the most common bacterial isolates
    identified and time to detection (TTD).
• Retrospective data analysis of the sterility testing performed on a subset of
  apheresis platelets collected between 2011 and 2016.
• The sterility sample is collected aseptically in a steriley docked pouch.
  – 2 to 4 ml of platelet product is equally divided in an aerobic and anaerobic culture
    bottle using the BacT/ALERT (Biomerieux) or BD BACTEC™ system
  – Culture for 14 days.
Aim and Methods: Principle of BacT/ALERT Microbial Detection System

- If microorganisms are present in the test sample, CO2 is produced as the organisms metabolize the substrates in the culture medium.
- A colorimetric sensor and reflected light is used to monitor the presence and production of CO2 dissolved in the culture medium.
- Bottle reflectance is monitored continuously.
- The color of the gas-permeable sensor installed in the bottom of each culture bottle changes from blue-green to Yellow → Positive growth signal → Bacterial identification.
Results

Number of AP Procedures carried out per month and year

- Average annual collections range from 14,000 to 15,000 (approx. 1,200/month)
- Slight downward trend over the years
Results
Proportion of Apheresis Collections submitted for sterility testing.

- Monthly numbers of AP tested for sterility has increased significantly over the last years.
- The average percentage of AP platelets tested annually was 18%,
- The proportion increased from 11% to 20%.
## Results

### % positive cultures per month

<table>
<thead>
<tr>
<th>Year</th>
<th>Plat Coll tested</th>
<th>QA tested</th>
<th>Ster Pos</th>
<th>% Ster Pos</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011</td>
<td>15,049</td>
<td>1,660</td>
<td>10</td>
<td>0.60 %</td>
</tr>
<tr>
<td>2012</td>
<td>15,408</td>
<td>2,767</td>
<td>26</td>
<td>0.94 %</td>
</tr>
<tr>
<td>2013</td>
<td>14,794</td>
<td>2,777</td>
<td>61</td>
<td>2.20 %</td>
</tr>
<tr>
<td>2014</td>
<td>14,402</td>
<td>2,714</td>
<td>42</td>
<td>1.55 %</td>
</tr>
<tr>
<td>2015</td>
<td>14,209</td>
<td>2,852</td>
<td>149</td>
<td>5.22 %</td>
</tr>
<tr>
<td>2016</td>
<td>13,981</td>
<td>2,860</td>
<td>141</td>
<td>4.93 %</td>
</tr>
<tr>
<td><strong>Grand Total</strong></td>
<td><strong>87,843</strong></td>
<td><strong>15,630</strong></td>
<td><strong>429</strong></td>
<td><strong>2.74 %</strong></td>
</tr>
</tbody>
</table>

- Annual rates ranged between 0.6% in 2011 to 5.22% in 2015.
- The positive rates were consistently higher in the summer months.
- 3.3% to 4.5% in October to January vs to 1.2% to 2.3% between February to July.
### Results: Frequency of Bacteria identified and TTP (days)

<table>
<thead>
<tr>
<th>Genus spp.</th>
<th>Frequency</th>
<th>Percentage</th>
<th>TTP*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propionebacterium spp.</td>
<td>90</td>
<td>33%</td>
<td>7,2</td>
</tr>
<tr>
<td>Staphylococcus spp.</td>
<td>74</td>
<td>27%</td>
<td>3,9</td>
</tr>
<tr>
<td>Bacillus spp.</td>
<td>24</td>
<td>9%</td>
<td>6,5</td>
</tr>
<tr>
<td>Corynebacterium spp.</td>
<td>21</td>
<td>8%</td>
<td>4,5</td>
</tr>
<tr>
<td>Micrococcus spp.</td>
<td>18</td>
<td>7%</td>
<td>3,6</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>13</td>
<td>5%</td>
<td>7,8</td>
</tr>
<tr>
<td>Other (21 organisms)</td>
<td>30</td>
<td>11%</td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>270</strong></td>
<td></td>
<td><strong>5,9</strong></td>
</tr>
</tbody>
</table>

* Time to Positivity was not available for all isolates

<table>
<thead>
<tr>
<th>Pathogenic isolates</th>
<th>Frequency</th>
<th>TTP*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Listeria grayi</em></td>
<td>3</td>
<td>5,5</td>
</tr>
<tr>
<td><em>Acinetobacter</em> spp.</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td><em>Enterobacter</em> spp.</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td><em>Serratia liquefaciens</em></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>9</strong></td>
<td><strong>3,25</strong></td>
</tr>
</tbody>
</table>

- 80% of bacteria are skin commensals
- 3% were true pathogens (5/9 GNB)
- Pathogens identified earlier than skin / environmental commensals
- *P.acnes* was preferentially isolated anaerobically.
Conclusions

• There is a trend of an increasing positive sterility rates between 2011 and 2016.

• Direct comparisons with international data cannot be done as SANBS methods are not fully aligned and positive results are not confirmed.

• No reports of sepsis or death have been reported to SANBS in this time, but we assume such events to be underreported.

• In agreement with other publications Gram positive skin commensals account for 80% of all bacteria isolated.

• Due to limited AP stock, issuing of platelets early (by Day 3) is a good risk mitigation strategy.
Conclusions

• Interventions made:
  – Infection control practises have been strengthened across the value chain
  – Monthly AP sterility rates / donation site are tracked and communicated
  – Sterility testing practises need to be tighter aligned with international recommendations
    • We have increased our culture volume to minimum amount using aerobic bottle only
    • Validating the value of reducing our culturing time.
    • Perform sterility testing on pooled platelets

• Future Interventions to be considered to produce safer platelet products for SA patients:
  • Pathogen inactivation
  • Pre-release platelet testing Day 4 and Day 5.
Acknowledgements:

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