In vitro Comparison of q-FGP and pi-FGP (Intercept)

Dr. David Goslings – Head of Production at Blood Transfusion Service Zurich, SRC
# Types of Plasma for Transfusion Available in Switzerland

<table>
<thead>
<tr>
<th></th>
<th>Quarantine-stored Fresh Frozen Plasma</th>
<th>Intercept Treated Fresh Frozen Plasma</th>
<th>OctaplasLG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbreviation</td>
<td>q-FFP</td>
<td>pi-FFP</td>
<td>S/D-Plasma</td>
</tr>
<tr>
<td>Pathogen Inactivated (PI)</td>
<td>no</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Method of PI</td>
<td>-</td>
<td>Photochemical Treatment</td>
<td>Solvent/Detergent Treatment</td>
</tr>
<tr>
<td>Pool Size</td>
<td>1 donation</td>
<td>1 - 6 donations</td>
<td>≈380 - 1500L</td>
</tr>
<tr>
<td>Volume per Unit</td>
<td>260 mL (in average)</td>
<td>≈ 200 mL</td>
<td>≈200 mL</td>
</tr>
<tr>
<td>Factor VIII per Unit</td>
<td>≥ 0.7 IU/mL</td>
<td>≥ 0.5 IU/mL</td>
<td>≥ 0.5 IU/mL</td>
</tr>
</tbody>
</table>
Both pathogen inactivation methods reduce certain proteins of the coagulation pathway.

So far, there is a tradeoff when it comes to pathogen inactivation of plasma.

However, is this tradeoff relevant?

<table>
<thead>
<tr>
<th>Protein</th>
<th>Untreated</th>
<th>SD</th>
<th>Amotosalen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen</td>
<td>100</td>
<td>91</td>
<td>81</td>
</tr>
<tr>
<td>Factor V</td>
<td>100</td>
<td>65</td>
<td>96</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>100</td>
<td>70</td>
<td>75</td>
</tr>
<tr>
<td>Factor XI</td>
<td>100</td>
<td>86</td>
<td>89</td>
</tr>
<tr>
<td>ADAMTS 13</td>
<td>100</td>
<td>100</td>
<td>98</td>
</tr>
<tr>
<td>Protein S</td>
<td>100</td>
<td>57</td>
<td>95</td>
</tr>
<tr>
<td>Antiplasmin</td>
<td>100</td>
<td>20</td>
<td>85</td>
</tr>
</tbody>
</table>
Effect of Intercept on Apheresis-Plasma

Recovery [%] 77

Extrinsic Pathway

Inhibitors

Recovery [%] (Intrinsic Pathway)

INTERCEPT plasma: comparability with conventional fresh-frozen plasma based on coagulation function – an in vitro analysis

Clinical Studies on Intercept Plasma

HEMAPHERESIS

A randomized, controlled Phase III trial of therapeutic plasma exchange with fresh-frozen plasma (FFP) prepared with amotosalen and ultraviolet A light compared to untreated FFP in thrombotic thrombocytopenic purpura


CONCLUSION: The comparable results between treatment groups observed from this small trial suggest that TPE with PCT FFP was safe and effective for treatment of TTP.

BLOOD COMPONENTS

Comparative effectiveness of plasma prepared with amotosalen-UVA pathogen inactivation and conventional plasma for support of liver transplantation

Jacques Cinqualbre,1 Daniel Kientz,2 Emilie Remy,2 Norman Huang,3 Laurence Corash,3 and Jean Pierre Cazenave4

TRANSFUSION 2015;55;1710–1720

CONCLUSION: In this retrospective study, IBS plasma provided therapeutic support of liver transplant not different from Q-FFP.

Photochemically treated fresh frozen plasma for transfusion of patients with acquired coagulopathy of liver disease

Paul D. Mintz, Nathan M. Bass, Lawrence D. Petz, Randolph Steadman, Michael Streiff, Jeffery McCullough, Sandra Burks, David Wages, Sally Van Doren, and Laurence Corash

... These results suggest PCT-FFP supported hemostasis in the treatment of acquired coagulopathy similarly to conventional FFP. (Blood. 2006;107:3753-3760)
CAT Assay

The three pathways that makeup the classical blood coagulation pathway

**Intrinsic**

- Surface contact
  - XII → XIIₐ
  - XI → XІₐ
  - IX → IXₐ

**Extrinsic**

- Tissue damage
  - TF: VIIₐ

**Common**

- Prothrombin → Thrombin (serine protease)
  - (V, PL, Ca²⁺)
  - (VIII, PL, Ca²⁺)

**CAT Assay**

- Fibrinogen → Fibrin
  - XII – Hageman factor, a serine protease
  - XI – Plasma thromboplastin, antecedent serine protease
  - IX – Christmas factor, serine protease
  - VII – Stable factor, serine protease
  - XIII – Fibrin stabilising factor, a transglutaminase
  - PL – Platelet membrane phospholipid
  - Ca²⁺ – Calcium ions
  - TF – Tissue Factor (ₐ = active form)

Thrombinogram of the CAT Assay

- Addition of Tissue-Faktor (TF) und phospholipids start thrombin generation.
- Thrombin binds to a substrat causing fluorescence that can be used for quantification of thrombin generation.
- ETP (Endogenous Thrombin Potential) is the most important parameter because it is proportional to the amount of thrombin activity formed.
- The amount of TF added has a large influence on the fluorescence reaction (e.g. coagulation factor specify). Standard concentrations are 1 or 5 pM TF, physiological concentration are probably > 5pM.

Effects of Intercept on Pooled Recovered Plasma

**Factor Recovery after PI**

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Value (av.)</th>
<th>Reference Range*</th>
<th>Recovery (av.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen [g/L]</td>
<td>2.2</td>
<td>1.7 - 4.0</td>
<td>79%</td>
</tr>
<tr>
<td>Factor V [%]</td>
<td>78</td>
<td>70 - 120</td>
<td>102%</td>
</tr>
<tr>
<td>Factor VIII [%]</td>
<td>75</td>
<td>50 - 150</td>
<td>70%</td>
</tr>
<tr>
<td>Factor XIII [%]</td>
<td>105</td>
<td>70 - 140</td>
<td>92%</td>
</tr>
<tr>
<td>Antithrombin [%]</td>
<td>80</td>
<td>80 - 140</td>
<td>89%</td>
</tr>
<tr>
<td>Protein S [%]</td>
<td>87</td>
<td>55 - 140</td>
<td>90%</td>
</tr>
<tr>
<td>Antiplasmin [%]</td>
<td>72</td>
<td>80 - 150</td>
<td>84%</td>
</tr>
<tr>
<td>VWF [%]</td>
<td>84</td>
<td>50 - 200</td>
<td>100%</td>
</tr>
<tr>
<td>ADAMTS13 [%]</td>
<td>80</td>
<td>51 - 100</td>
<td>88%</td>
</tr>
</tbody>
</table>

**ETP before and after PI**

*95% Confidence Interval for the Mean*

![Thrombinogram Example](image)


n=5 except for ADAMTS13 (n=1)
‘It’s not the average that kills you, it’s the variability’

MB-Plasma withdrawal in France

Q. What official reasons did AFSSAPS* give for their decision to withdraw THERAFLEX MB-Plasma from the list of approved labile blood products in France?

1) ...

2) a greater variability in the concentration of fibrinogen in MB treated plasma compared to the other types of treated plasma.


http://web.stanford.edu/~savage/flaw/
The INTERCEPT Blood System for Plasma

Process entry volume: 385 – 650ml

Volume final product: 200mL

Step 1: Sterile docking
Step 2: Amotosalen
Step 3: Illumination
Step 4: CAD
Step 5: Partitioning

Blood Transfusion Service Zurich, SRC
Pooling Effect

- Sterile connect 5 units of filtered recovered plasma (Ø ≈ 260mL)
- Pool of 5 units of recovered plasma (5x ≈ 260mL ≈ 1300mL)
- Receive 2 splits of 630 – 650mL each
- Sterile connect each split to a INTERCEPT Set
- Receive 3 units pi-FFP per Intercept Set (200ml/unit)

Blood Transfusion Service Zurich, SRC
Pooling Effect

Fibrinogen
Single Value Diagram

Blood Transfusion Service Zurich, SRC
Pooling Effect

Single Value Diagram

- Factor V
- Factor VIII
- Factor XIII
- Antithrombin
- Protein S
- Antiplasmin
- VWFA
- ADAMTS 13

- Single unit before PI
- Pool before PI
- Pool after PI

Blood Transfusion Service Zurich, SRC
### Impact of INTERCEPT on proteins of the coagulation system

- Intercept treatment reduces activity of some but not all factors of the coagulation pathway (Recovery between 70 and 100%)
- In general, factors are still within the physiological reference range after intercept treatment.
- Pooling plasma units prior to pathogen inactivation reduces the natural variability of factor concentrations.

### Impact of INTERCEPT on *in vitro* Thrombin Generation (CAT-Assay)

- ETP is the most important parameter of the CAT-Assays.
- Average ETPs of pi-FFP and q-FFP from whole blood are perhaps not significantly different. This may be an explanation for the good clinical experiences with Intercept-FFP, although some factor activity reduction clearly occurs. Further investigations are necessary.

### Outlook

Many questions are still not answered and one of them has been addressed in a cooperation between USZ and ZHBSD: Is there a difference between Swiss Intercept-Plasma and Swiss quarantine stored plasma from whole blood with respect to coagulation factors and global coagulation capacity (CAT Assay)? The study will not only capture the impact pathogen inactivation but also other quality relevant effects like different processing times of the two plasma qualities.
Reduction of Factor VIII during Storage at RT

<table>
<thead>
<tr>
<th>Plasma ID</th>
<th>Duration of Storage [hh:mm]</th>
<th>F VIII at Start [IU/mL]</th>
<th>F VIII at Stop [IU/mL]</th>
<th>F VIII Loss [IU/mL]</th>
<th>relative</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>10:35</td>
<td>0.97</td>
<td>0.80</td>
<td>0.17</td>
<td>17.5%</td>
</tr>
<tr>
<td>b</td>
<td>10:35</td>
<td>0.54</td>
<td>0.46</td>
<td>0.08</td>
<td>14.8%</td>
</tr>
<tr>
<td>c</td>
<td>10:35</td>
<td>0.90</td>
<td>0.69</td>
<td>0.21</td>
<td>23.3%</td>
</tr>
<tr>
<td>d</td>
<td>10:10</td>
<td>1.42</td>
<td>1.20</td>
<td>0.22</td>
<td>15.5%</td>
</tr>
<tr>
<td>e</td>
<td>10:10</td>
<td>1.08</td>
<td>0.88</td>
<td>0.20</td>
<td>18.5%</td>
</tr>
<tr>
<td>f</td>
<td>10:35</td>
<td>0.76</td>
<td>0.67</td>
<td>0.09</td>
<td>11.8%</td>
</tr>
<tr>
<td>g</td>
<td>10:35</td>
<td>0.72</td>
<td>0.62</td>
<td>0.10</td>
<td>13.9%</td>
</tr>
<tr>
<td>h</td>
<td>10:25</td>
<td>1.04</td>
<td>0.88</td>
<td>0.16</td>
<td>15.4%</td>
</tr>
<tr>
<td>i</td>
<td>10:25</td>
<td>0.79</td>
<td>0.65</td>
<td>0.14</td>
<td>17.7%</td>
</tr>
<tr>
<td>j</td>
<td>10:25</td>
<td>0.79</td>
<td>0.66</td>
<td>0.13</td>
<td>16.5%</td>
</tr>
<tr>
<td>Av</td>
<td>10:27</td>
<td>0.90</td>
<td>0.75</td>
<td>0.15</td>
<td>16.5%</td>
</tr>
<tr>
<td>Sdv</td>
<td></td>
<td>0.24</td>
<td>0.20</td>
<td>0.05</td>
<td>3.11%</td>
</tr>
<tr>
<td>CV</td>
<td></td>
<td>27.01</td>
<td>26.92</td>
<td>33.70</td>
<td>18.84</td>
</tr>
<tr>
<td>Med</td>
<td>10:30</td>
<td>0.85</td>
<td>0.68</td>
<td>0.15</td>
<td>15.97%</td>
</tr>
<tr>
<td>Min</td>
<td>10:10</td>
<td>0.54</td>
<td>0.46</td>
<td>0.08</td>
<td>11.84%</td>
</tr>
<tr>
<td>Max</td>
<td>10:35</td>
<td>1.42</td>
<td>1.20</td>
<td>0.22</td>
<td>23.33%</td>
</tr>
</tbody>
</table>
Study Design

Purpose:
In Vitro comparison of Swiss recovered q-FFP and pi-FFP manufactured and stored under routine conditions at the same centre. Not only the effect of Intercept treatment will be captured but also other production related effects like e.g. storing plasma units over night. Furthermore, to consider the worst case scenario concerning F VIII, exclusively FFPs from donors with blood group 0 will be included into the study.

Method:
35 q-FFPs and 35 pi-FFPs of comparable age from male donors of blood group 0 will be investigated as follows:
- Fibrinogen (accord. to Clauss)
- Fibrinogen (ag)
- Factor V (funct.)
- Factor VIII (funct.)
- Factor XIII (funct.)
- Von Willebrand Factor (funct.)
- CAT Assay
- Antithrombin (funct.)
- Protein S (ag. free)
- Antiplasmin (funct.)
- ADAMTS-13 (funct.)
- ROTEM
In vitro Study on Intercept-FFP vs q-FFP

Title:
QUARANTINE VERSUS PATHOGEN REDUCED PLASMA - COAGULATION FACTOR CONTENT AND ROTATIONAL THROMBOELASTOMETRY COAGUALTION

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Thanks for your attention...