EDQM SYMPOSIUM

PLASMA FOR DIRECT CLINICAL USE

22-23 SEPTEMBER 2015, STRASBOURG, FRANCE

SLIDES

Session 2
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Novel component: liquid plasma thawed or non-frozen

Exsanguination accounts for ~30% of the mortality in trauma

Blood products (RBC, Plasma, Platelets) are required immediately
Transfusion practice in massively bleeding patients: time for a change?

P.T. Johansson, M. B. Haveron & H. Sonesson
Department of Clinical Hematology, University Hospital of Copenhagen, Copenhagen, Denmark

Background and Objectives. We identified some fatal cases where massively bleeding patients received inadequate transfusion therapy. The aim of this study was to review and evaluate the transfusion practice in acutely multi-traumatized patients.

Materials and Methods. Patients receiving > 10 units of red blood cells (RBC) within 24 h of admission and ≥ 30 blood components within 7 days of admission were reviewed.

Results. Thirty-nine patients were identified, 13 of whom were inadequately transfused (IT) and had a higher mortality (12/13) than adequately transfused (AT) patients 13/26 (P = 0.013). Ten of 13 IT patients developed a microvascular bleed compared to four of 26 in the AT group (P = 0.001) and had a lower platelet count upon arrival at the intensive care unit (40 × 10^9/l vs. 80 × 10^9/l, P = 0.04). Conclusions. An early balanced transfusion therapy is vital in massively bleeding patients, and a pro-active approach from the blood bank is warranted. We have introduced an acute transfusion package (ATP) consisting of 6 RBC, 5 FFP and 2 PC units, indicated in massively bleeding patients, securing a balanced transfusion therapy.

Key words: bleeding, blood components, transfusion.

Quality control study

- Thawed plasma units (pre-stored & not pre-stored)
- Measurements for 72 hours
- FVIII, FV, Fibrinogen
- Based on these data we introduced thawed FFP stored for up to 72 hours to be used in the transfusion packages
- The same patients were allowed to receive a maximum of 2 transfusion packages with stored thawed FFP

Experience with pre-thawed FFP

- Between 2004 – 2009 1.445 patients received 1.965 transfusion packages encompassing 9826 units of pre-thawed FFP
- Each patient on average received 1.3 transfusion packages
- 91 patients received more than 5 transfusion packages
- The maximum amount of transfusion packages administered per patient was 10
- The discard rate was 1.7%
Transfusion packages for massively bleeding patients:
The effect on clot formation and stability as evaluated by Thrombelastograph (TEG®)

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bDepartment of Anaesthesiology, Rigshospitalet, University of Copenhagen, Denmark

Abstract

We investigated the effect of administering a transfusion package encompassing 5 red blood cells (RBCs), 5 fresh frozen plasma (FFP), and 2 platelet concentrates (PC) on clot formation and stability as evaluated by Thrombelastograph (TEG®) in 10 patients presenting with massive bleeding. Blood was obtained before and after administering the transfusion packages. Six patients were hypocoagulable before administration of the transfusion package, whereas none of the patients were hypocoagulable after transfusion of up to 7 transfusion packages (p < 0.01). In 5 patients damage control surgery was successful and 6 of these patients survived. The result indicates that an early balanced transfusion strategy maintains haemostatic competence in massively bleeding patients.

Viscoelastical haemostatic assays (VHA)

- Whole blood analysis
- Measures the viscoelastic properties of the clot
- Global assessment of clot build up and degradation
- Rapid tests (15 min.)

TEG®

ROTEM®
Transfusion packages and TEG

<table>
<thead>
<tr>
<th>Patients (No.)</th>
<th>10</th>
<th>10</th>
<th>8</th>
<th>5</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transfusion package (&quot;TP; No.&quot;)</td>
<td>0 (Before TP)</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>R time (3-8 min)</td>
<td>6.7 (3.7-9.4)</td>
<td>6.0 (4.8-7.6)</td>
<td>6.4 (4.9-7.5)</td>
<td>6.5 (5.0-7.2)</td>
<td>6.8</td>
</tr>
<tr>
<td>Angle (°) (55-78°)</td>
<td>58.9 (48.1-71.5)</td>
<td>*63.3 (53.1-72.4)</td>
<td>*61.3 (55.2-69.2)</td>
<td>60.8 (55.8-67.3)</td>
<td>60.3</td>
</tr>
<tr>
<td>MA (51-69 mm)</td>
<td>48.9 (44.3-63.7)</td>
<td>*59.3 (56.5-65.9)</td>
<td>*57.0 (53.4-63.1)</td>
<td>*56.2 (52.2-59.7)</td>
<td>*55.3</td>
</tr>
<tr>
<td>G (4.6-10.9 d/sc)</td>
<td>4.8 (4.0-8.8)</td>
<td>*7.3 (4.6-9.6)</td>
<td>*7.1 (5.0-8.8)</td>
<td>*7.0 (5.2-8.3)</td>
<td>*6.8</td>
</tr>
<tr>
<td>Ly30 (0-8%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hypocoagulable patients (No.)</td>
<td>6</td>
<td>0**</td>
<td>0**</td>
<td>0**</td>
<td>0**</td>
</tr>
</tbody>
</table>

Johansson et al. Transf Apher Sci. 2008;39:3-8

Change

- In 2010 the Danish Health and Medicines Authority decided that pre-thawed plasma only could be stored for 24 hours
- This was immediately implemented and resulted in an increase in discard rates from 1.7% to 4.8%

EDQM Symposium on "Plasma for Direct Clinical Use", 22-23 Sept. 2015
Physician staffed CPH HEMS since 2010

- 2 RBCs O Rh D Negative
- Logger from the Blood Bank

HEMS CPH 2011-2013

- 35 trauma patients received pre-hospital RBCs
- Age mean 44 years
- ISS < 15: 9%, ISS 15-25: 18%, ISS > 25: 72%

- Transfusions in-hospital
  - RBCs 13
  - FFP 12
  - PC 4
  - Waste: 6%

- Mortality 24 h: 29%

Thomsen AB, Jørgensen H, Salado J, Johansson PI. Aired Conf 2014
Life Flight

- Houston, TX, USA
- 2 RBCs + 2 thawed FFP (< 5 days)

### Multivariate logistic regression for mortality within the first 6 hours

<table>
<thead>
<tr>
<th></th>
<th>Odds Ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEMS</td>
<td>0.23</td>
<td>0.033</td>
</tr>
<tr>
<td>Arrival base deficit</td>
<td>0.87</td>
<td>0.003</td>
</tr>
<tr>
<td>Arrival rTEG ACT</td>
<td>1.02</td>
<td>0.035</td>
</tr>
<tr>
<td>Arrival SBP</td>
<td>0.93</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Blunt mechanism of injury</td>
<td>0.29</td>
<td>0.050</td>
</tr>
<tr>
<td>Prehospital crystalloid, mL</td>
<td>0.99</td>
<td>0.637</td>
</tr>
</tbody>
</table>

Holcomb JB et al. PREHOSPITAL EMERGENCY CARE 2015

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The impact of plasma preparations and their storage time on short-term posttransfusion mortality: A population-based study using the Scandinavian Donation and Transfusion database

Rat Nords, MD, PhD, Therese M-L. Andersson, MSc, Gustaf Edgren, MD, PhD, Olof Nygren, MD, PhD, and Marie Reilly, PhD, Stockholm, Sweden

**BACKGROUND:** The treatment of transfusiology and bleeding in severe trauma requires rapid delivery of large amounts of plasma in emergency wards. The resulting need for adequate supplies of fresh or thawed plasma has consequences for storage strategies. Using extensive population data from a cohort of 64,454 Swedish patients followed up from first recorded allogeneic plasma transfusion for 365 days post-transfusion, this study investigates whether there is an association between short-term mortality after receipt of TTP or liquid plasma of different storage times.

**METHODS:** A cohort of 64,454 Swedish patients was followed up from first recorded allogeneic plasma transfusion for 365 days post-transfusion. Associations with exposure to TTP were assessed as relative risks adjusted for patient characteristics, crude transaminas, transfusion, and calendar year. For post-TPR, the aim was to estimate the risk of death from a TTP for each patient.

**RESULTS:** The relative risk of exposure to TTP was 1.5 (95% confidence interval: 1.2-1.9, p < 0.0001), with the risk elevation continued to the end of the calendar year of the study. There was an increase of only 0.5% of mortality per day of the TTP. In analysis of all plasma types, TTP from male donors had lower risk.

**CONCLUSIONS:** Compared with non-use of risk-free plasma, TTP was associated with increased cardiovascular mortality in the in-hospital mortality window. TTP from female donors had a significantly higher risk than each TTP. Post-TPR, plasma of storage time was associated with mortality. These findings can help in informing policies for managing high plasma demand in critical care. (J Thromb Thrombolysis 2012;33:94-101. Copyright © 2012 by Lippincott Williams & Wilkins)
TABLE 3. Adjusted* Estimates of Relative Risk Associated with Exposure to FFP among Adult Swedish Recipients of Any Plasma Product at Their Index† Transfusion Episodes in 1990–2002

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Relative Risk (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Only non-frozen plasma</td>
<td>1.00 (ref)</td>
<td></td>
</tr>
<tr>
<td><strong>At least one unit of FFP</strong></td>
<td>1.19 (1.12–1.27)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Units FFP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.00 (ref)</td>
<td></td>
</tr>
<tr>
<td>1–2</td>
<td>1.16 (1.09–1.24)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>3–4</td>
<td>1.19 (1.09–1.29)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>5–10</td>
<td>1.35 (1.22–1.49)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>11+</td>
<td>1.45 (1.24–1.68)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

* Adjusted for blood group, sex and age (18–20, 21–25, 26–30, …, 86–90, 91+) of recipient, hospital and calendar year of transfusion, number of RBC units (0, 1–2, 3–4, 5–9, 10+) received during follow-up and in the 36 days prior, total number of plasma units of any kind (on a log-linear scale) received during follow-up, time (in days) since the start of follow-up, and the indication for transfusion in six broad categories (see text).

†The first allogeneic plasma transfusion after January 1, 1990, recorded in the Scandinavian Donation and Transfusion (SCANDAT) database.

The reference category consists of patients who were exclusively exposed to non-FFP plasma.

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**Liquid plasma study in CPH**

[Diagram of a plasma pose with measurements and labels]
Liquid plasma

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total protein g/l</th>
<th>Fibrinogen μmol/l</th>
<th>vWF 10^9/ml enh./l</th>
<th>PT 10^9 arb. enh./l</th>
<th>FVII 10^9 arb. enh./l</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>b</td>
<td>a</td>
<td>b</td>
<td>b</td>
<td>b</td>
</tr>
<tr>
<td>Day 0</td>
<td>59.9 (25.0-65.6)</td>
<td>7.45 (6.06-8.69)</td>
<td>0.89 (0.66-1.13)</td>
<td>0.69 (0.52-1.0)</td>
<td>1.34 (0.76-1.27)</td>
</tr>
<tr>
<td>Day 1</td>
<td>55.3 (29.0-60.8)</td>
<td>7.35 (6.06-8.20)</td>
<td>1.0 (0.62-1.2)</td>
<td>0.83 (0.66-1.11)</td>
<td>0.71 (0.11-0.49)</td>
</tr>
<tr>
<td>Day 3</td>
<td>58 (56.0-60.6)</td>
<td>6.8 (6.44-8.40)</td>
<td>0.81 (0.67-1.15)</td>
<td>0.73 (0.55-1.11)</td>
<td>0.6 (0.4-0.95)</td>
</tr>
<tr>
<td>Day 7</td>
<td>66.8 (54.0-61.0)</td>
<td>8.2 (5.89-9.80)</td>
<td>0.75 (0.62-1.23)</td>
<td>0.75 (0.66-0.96)</td>
<td>0.74 (0.63-0.86)</td>
</tr>
<tr>
<td>Day 10</td>
<td>58.5 (55.0-61.0)</td>
<td>7.7 (6.3-9.6)</td>
<td>0.79 (0.62-1.0)</td>
<td>0.78 (0.66-0.80)</td>
<td>0.79 (0.63-0.86)</td>
</tr>
<tr>
<td>Day 14</td>
<td>58.5 (55.0-61.0)</td>
<td>7.8 (7.8-9.7)</td>
<td>0.9 (0.61-1.32)</td>
<td>0.74 (0.64-0.91)</td>
<td>0.9 (0.63-0.91)</td>
</tr>
</tbody>
</table>

$\Delta^\prime$ (%) 0-14 dg -3.47 0.98 1.51 -18.14 -45.01

p* 0.005 0.04 0.43 <0.0001 <0.00001

# Median-værdier og range

* Beregnet som gennemsnit af alle parvise ændringer. Negativt fortegn angiver et fald, positivt fortegn en stigning.

* Wilcoxon signed rank sum test.

Liquid plasma TEG

<table>
<thead>
<tr>
<th>Parameter</th>
<th>R-tid (FF) min</th>
<th>Angle (FF) deg</th>
<th>MA (FF) mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>b</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>Day 0</td>
<td>1.10 (0.90-1.20)</td>
<td>73.65 (68.3-77)</td>
<td>74.4 (17.5-30.1)</td>
</tr>
<tr>
<td>Day 1</td>
<td>1.00 (0.90-1.20)</td>
<td>74.95 (72.2-79)</td>
<td>74.85 (20.0-31.5)</td>
</tr>
<tr>
<td>Day 3</td>
<td>1.20 (1.09-1.30)</td>
<td>74.7 (72.7-77.8)</td>
<td>25.19 (21.1-39.0)</td>
</tr>
<tr>
<td>Day 7</td>
<td>1.30 (1.29-1.50)</td>
<td>65.55 (55.5-73)</td>
<td>23.18 (16.1-39.4)</td>
</tr>
<tr>
<td>Day 10</td>
<td>1.20 (1.19-1.40)</td>
<td>72.45 (69.2-72.8)</td>
<td>24.4 (19.8-30.6)</td>
</tr>
<tr>
<td>Day 14</td>
<td>1.30 (1.19-1.50)</td>
<td>71.22 (60.3-75.1)</td>
<td>24.48 (17.5-33.2)</td>
</tr>
</tbody>
</table>

$\Delta^\prime$ (%) 0-14 dg 30.35 -7.13 3.89

p* 0.002 0.024 0.63
Liquid plasma is available in CPH HEMS since January 2014

Prehospital Resuscitation On Helicopter Study (PROHS)

1. National HEMS, Rigshospitalet, Copenhagen, Denmark
2. Memorial Hermann Hospital/University of Texas Health Science Center at Houston
3. University Hospital Cincinnati
4. Mayo Medical Center
5. Oregon Health and Science University
6. Harborview Medical Center
7. University of Maryland Medical Center
8. LA County/University of Southern California Medical Center
9. University of Alabama – Birmingham
10. University of Arizona
Thank you

Some nice, fresh blood ... This will get you up and running in no time ...
Novel component: freeze-dried plasma

Christophe Martinaud (MD, PhD)
French Military Blood Institute
Clamart, France

Strasbourg, mardi 22 septembre 2015

OVERVIEW

1. Lyophilized plasma available
2. French Lyophilized Plasma (FLYP) history
3. Main characteristics of FLYP
4. Internal & external in vitro quality controls
5. Stability studies
6. Clinical and biological data from 2010
7. Perspectives
OVERVIEW

1. Lyophilized plasma available
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7. Perspectives

Lyophilized plasma available

- South-Africa = Bioplasma®
  - pool of unpaid donors
  - SD treated
  - universal blood group compatibility
  - national use
  - few studies in cardiac surgery

<table>
<thead>
<tr>
<th>Factors</th>
<th>1 unit</th>
<th>2 units</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bio</td>
<td>Fdp</td>
</tr>
<tr>
<td>V</td>
<td>0.2 (0.04 - 0.33)</td>
<td>0.3 (0.06 - 0.83)</td>
</tr>
<tr>
<td>VII</td>
<td>0.8 (0.4 - 1.3)</td>
<td>1.4 (0.2 - 4.1)</td>
</tr>
<tr>
<td>VIII</td>
<td>0.9 (0.4 - 1.7)</td>
<td>2.4 (0.8 - 3.6)</td>
</tr>
<tr>
<td>IX</td>
<td>0.5 (0.2 - 0.7)</td>
<td>0.6 (0.2 - 1.1)</td>
</tr>
<tr>
<td>X</td>
<td>0.7 (0.4 - 1.2)</td>
<td>0.8 (0.2 - 1.6)</td>
</tr>
</tbody>
</table>
Lyophilized plasma available

- South-Africa = Bioplasma®
- Germany = LyoPlas N-W®
  - apheresis plasma
  - securized by quarantine

OVERVIEW

1. Lyophilized plasma available
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Freeze Dried Plasma: history

1942
Transfusion in the point-of- wounding care by J. Juillard
First French use of FDP provided by the US Army

1946-1954
Indochina war: >30,000 lyophilized plasma infused

1949
FMBl: lyophilized plasma production

1951
Production of lyophilized plasma obtained from single donor, secured by quarantine process

1985
Blood contamination scandal in France: leading to a complete stop in pooled lyophilized plasma

1991
Gulf War: production of lyophilized plasma obtained from single donor, secured by quarantine process

1994
Pooled FDP is quarantine for 60 days prior to delivering

1994
Pathogen inactivation: French Lyophilized Plasma (FLyP)

OVERVIEW

1. Lyophilized plasma available
2. French Lyophilized Plasma (FLyP) history
3. Main characteristics of FLyP
4. Internal & external in vitro quality controls
5. Stability studies
6. Clinical and biological data from 2010
7. Perspectives
FLyP: an apheresis issued product

Donors are all volunteers and undergo a rigorous medical selection

Amotosalen based pathogen attenuation
FLyP: blood donation controls

- **All blood products**
  - Hemoglobin
  - ABO Rh Kell
  - HIV 1&2 antibodies & PCR
  - HCV antibodies & PCR
  - HBV antigen & PCR
  - HTLV antibodies
  - Syphilis antibodies

- **Some blood products**
  - Chagas antibodies
  - Malaria antibodies

- **Only for plasma**
  - HLA antibodies
  - Hemostasis tests
  - HEV PCR

FLyP: lyophilization - cryodessication
Description of FLyP

- Universal blood group compatibility
- pH=8 prior to administration
- 2-year shelf life at RT
- Reconstitution time < 6 min
- Therapeutic plasma monitored by the active hemovigilance & specific traceability
OVERVIEW

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7. Perspectives

FLyP: manufacturing process

<table>
<thead>
<tr>
<th>Mandatory release criteria</th>
<th>Additionnal tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>No hemolysins</td>
<td>Prothrombin time</td>
</tr>
<tr>
<td>Anti-A &amp; anti-B &lt; 1/64</td>
<td>Activated Partial Thromboplastin time</td>
</tr>
<tr>
<td>No irregular agglutinins</td>
<td>FV, FXI, FXIII</td>
</tr>
<tr>
<td>FVIII &gt; 0.5UI/mL</td>
<td>Protein C, Protein S, AT, α2-antiplasmin</td>
</tr>
<tr>
<td>Fibrinogen &gt; 2g/L</td>
<td>Plasma proteins electrophoresis</td>
</tr>
<tr>
<td>Residual amotosalen &lt; 2 µM</td>
<td>Thrombin Generation</td>
</tr>
<tr>
<td>Total proteins &gt; 50g/L</td>
<td>Viscoelastometry</td>
</tr>
<tr>
<td>Humidity &lt; 2%</td>
<td>TAT complex and F1+2</td>
</tr>
<tr>
<td>Sterility</td>
<td>pH</td>
</tr>
</tbody>
</table>
FLyP: *in vitro* properties

**In vitro properties of FLyP compared with other French plasmas**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Units</th>
<th>Solvent-Detergent-FFP</th>
<th>Intercept-FFP</th>
<th>Quarantine-FFP</th>
<th>FLyP</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen</td>
<td>g/L</td>
<td>2.8</td>
<td>2.7</td>
<td>2.8</td>
<td>2.4</td>
<td>2 - 4</td>
</tr>
<tr>
<td>Factor V</td>
<td>IU/mL</td>
<td>0.9</td>
<td>1.0</td>
<td>1.0</td>
<td>0.7</td>
<td>0.7 – 1.2</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>IU/mL</td>
<td>0.7</td>
<td>0.8</td>
<td>1.1</td>
<td>0.7</td>
<td>0.5 – 1.5</td>
</tr>
<tr>
<td>Factor XI</td>
<td>IU/mL</td>
<td>0.8</td>
<td>0.6</td>
<td>1.0</td>
<td>0.7</td>
<td>0.5 – 1.4</td>
</tr>
<tr>
<td>Protein C</td>
<td>IU/mL</td>
<td>1.0</td>
<td>0.9</td>
<td>1.2</td>
<td>0.9</td>
<td>0.7 – 1.2</td>
</tr>
<tr>
<td>Protein S</td>
<td>IU/mL</td>
<td>0.6</td>
<td>1.0</td>
<td>1.4</td>
<td>0.9</td>
<td>0.7 – 1.4</td>
</tr>
<tr>
<td>Antithrombin</td>
<td>IU/mL</td>
<td>0.9</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>0.8 – 1.2</td>
</tr>
<tr>
<td>α₂ antiplasmin</td>
<td>IU/mL</td>
<td>0.2</td>
<td>0.8</td>
<td>1.0</td>
<td>0.9</td>
<td>0.8 – 1.2</td>
</tr>
</tbody>
</table>

FLyP: *in vitro* properties

**Thrombin generation Assay**

*No relevant differences in the thrombin generation Especially when TF concentration increases*

Martinaud et al. Anesthesiology 2012
FLyP: *in vitro* model of fluid resuscitation
Clot initiation time

- FFP
- FLyP
- LR
- Baseline

Clot formation speed

- Alpha Angle (degrees)

- FFP
- FLyP
- LR
- Baseline

p=0.16

p=0.97

p=0.73
OVERVIEW

1. Lyophilized plasma available
2. French LYophilized Plasma (FLYP) history
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4. Internal & external in vitro quality controls
5. Stability studies
6. Clinical and biological data from 2010
7. Perspectives
After reconstitution

12 different FLYP from the same batch were studied

<table>
<thead>
<tr>
<th></th>
<th>20°C</th>
<th></th>
<th>4°C</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T 0</td>
<td>T + 2H</td>
<td>T + 6H</td>
<td>T + 24H</td>
<td>T 0</td>
<td>T + 2H</td>
<td>T + 6H</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>3,0</td>
<td>2,9</td>
<td>2,9</td>
<td>2,7</td>
<td>3,0</td>
<td>2,9</td>
<td>3,0</td>
</tr>
<tr>
<td>F VIII (UI/ml)</td>
<td>0,6</td>
<td>0,5</td>
<td>0,5</td>
<td>0,4</td>
<td>0,6</td>
<td>0,5</td>
<td>0,5</td>
</tr>
<tr>
<td>F V (%)</td>
<td>64,7</td>
<td>63,0</td>
<td>56,2</td>
<td>40,7</td>
<td>65,2</td>
<td>63,0</td>
<td>57,3</td>
</tr>
</tbody>
</table>

During 42 months storage

<table>
<thead>
<tr>
<th>Time (month)</th>
<th>0</th>
<th>27</th>
<th>42</th>
<th>0</th>
<th>27</th>
<th>42</th>
<th>0</th>
<th>27</th>
<th>42</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>4°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Humidity (%)</td>
<td>0,86</td>
<td>0,61</td>
<td>0,21*</td>
<td>1,21</td>
<td>0,89</td>
<td>0,2*</td>
<td>0,99</td>
<td>0,56</td>
<td>0,25*</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>2,15</td>
<td>2,22</td>
<td>2,27</td>
<td>2,53</td>
<td>2,68</td>
<td>2,79</td>
<td>2,43</td>
<td>2,60</td>
<td>2,64</td>
</tr>
<tr>
<td>F VIII (UI/ml)</td>
<td>0,69</td>
<td>0,79</td>
<td>0,6</td>
<td>0,81</td>
<td>0,82</td>
<td>0,78</td>
<td>0,75</td>
<td>0,78</td>
<td>0,74</td>
</tr>
<tr>
<td>F V (%)</td>
<td>64</td>
<td>67</td>
<td>67</td>
<td>77</td>
<td>74</td>
<td>76</td>
<td>72</td>
<td>71</td>
<td>71</td>
</tr>
<tr>
<td>Total Proteins (g/L)</td>
<td>55,9</td>
<td>58</td>
<td>56,4</td>
<td>51,9</td>
<td>57,4</td>
<td>59</td>
<td>53,0</td>
<td>56</td>
<td>61,3</td>
</tr>
</tbody>
</table>
### Stability studies in remote conditions

- **R1** - [28 - 53°C]  
  - Armored vehicle  
  - Tent & refrigerator

- **R2** - [4°C]  
  - Nurse bag  
  - Tent

- **R2** - [18 - 38°C]  
  - Tent

### Stability studies: results

**Mali & Rep. of Djibouti**

<table>
<thead>
<tr>
<th>TIME</th>
<th>3 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td></td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>0.88</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>2.47</td>
</tr>
<tr>
<td>F VIII (UI/ml)</td>
<td>0.76</td>
</tr>
<tr>
<td>F V (%)</td>
<td>64</td>
</tr>
<tr>
<td>Total proteins (g/L)</td>
<td>56.9</td>
</tr>
</tbody>
</table>
OVERVIEW

1. Lyophilized plasma available
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### Hemovigilance 2010 – 2014

#### 1868 FLYP transfused / 471 transfusion episodes / 386 recipients

<table>
<thead>
<tr>
<th>Gender of recipients</th>
<th>Other nationalities</th>
<th>France</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Female</strong></td>
<td>41</td>
<td>37</td>
<td>78</td>
<td>20,2%</td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td>172</td>
<td>127</td>
<td>299</td>
<td>77,5%</td>
</tr>
<tr>
<td><strong>Unknown</strong></td>
<td>7</td>
<td>2</td>
<td>9</td>
<td>2,3%</td>
</tr>
<tr>
<td><strong>Total with PLYO</strong></td>
<td>220</td>
<td>166</td>
<td>386</td>
<td>100,0%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Blood products transfused</th>
<th>Other nationalities</th>
<th>France</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FLYP only</strong></td>
<td>220</td>
<td>166</td>
<td>386</td>
<td>100,0%</td>
</tr>
<tr>
<td><strong>RBC and FLYP</strong></td>
<td>153</td>
<td>135</td>
<td>288</td>
<td>74,6%</td>
</tr>
<tr>
<td><strong>FWB and FLYP</strong></td>
<td>24</td>
<td>33</td>
<td>57</td>
<td>14,8%</td>
</tr>
</tbody>
</table>

---

#### Indications and context

<table>
<thead>
<tr>
<th>TANSFUSION INDICATION</th>
<th>ALL NATIONALITIES</th>
<th>TRANSFUSION CONTEXT</th>
<th>ALL NATIONALITIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemorrhagic shock</td>
<td>146 32,4%</td>
<td>Explosive weapon</td>
<td>78 18,0%</td>
</tr>
<tr>
<td>Active bleeding</td>
<td>108 23,9%</td>
<td>Blunt trauma</td>
<td>77 17,8%</td>
</tr>
<tr>
<td>Risk of bleeding</td>
<td>108 23,9%</td>
<td>Penetrating trauma</td>
<td>63 14,5%</td>
</tr>
<tr>
<td>Risk of coagulopathy</td>
<td>19 4,2%</td>
<td>Obstetric</td>
<td>4 0,9%</td>
</tr>
<tr>
<td>Not specified</td>
<td>70 15,5%</td>
<td>Not specified</td>
<td>211 48,7%</td>
</tr>
<tr>
<td>Total recipient</td>
<td>391 100,0%</td>
<td>Total recipient</td>
<td>391 100,0%</td>
</tr>
</tbody>
</table>
Hemovigilance 2010 – 2014

Coagulation testing

<table>
<thead>
<tr>
<th></th>
<th>PRIOR FLYP</th>
<th></th>
<th>AFTER FLYP</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>SD</td>
<td>Average</td>
<td>SD</td>
</tr>
<tr>
<td>PT (%)</td>
<td>46.9</td>
<td>19.4</td>
<td>50.9</td>
<td>18.2</td>
</tr>
<tr>
<td>Fibrinogene (g/L)</td>
<td>2.9</td>
<td>2.3</td>
<td>3.1</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Hemovigilance 2010 – 2014

Clinical evolution and tolerance

<table>
<thead>
<tr>
<th>Outcome</th>
<th>All nationalities</th>
<th>Clinical tolerance</th>
<th>All nationalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Favorable</td>
<td>197</td>
<td>Good</td>
<td>302</td>
</tr>
<tr>
<td>Not specified</td>
<td>137</td>
<td>Not specified</td>
<td>80</td>
</tr>
<tr>
<td>Aggravation</td>
<td>8</td>
<td>Adverse event</td>
<td>4</td>
</tr>
<tr>
<td>Death</td>
<td>44</td>
<td>Total recipient</td>
<td>386</td>
</tr>
<tr>
<td>Total recipient</td>
<td>386</td>
<td></td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Only 4 adverse events were reported in 5 years
- Fugace erythema
- Imputability is not sure
OVERVIEW

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Perspectives

- FLYP will be used in civilian area
  - In the emergency department (since 2013 in Lille)
  - In austere settings
  - In pre-hospital settings at the end of this year
Pre-hospital use

- Rational for plasma use asap
  - Borgman et al. J Trauma 2007
  - Holcomb et al. PROMMT Study - JAMA 2013

- Rational for plasma in pre-hospital
  - Karam O et al. Cochrane Database 2013

- Freeze dried plasma (LyoPlas)
  - Sunde et al. J Trauma Acute Care Surg 2014

France: PREHO – PLOY

- Criteria of inclusion
  - trauma patients with hemorrhage
  - PAS < 90 mmHg AND CF >108 bpm OR PAS < 70 mmHg

- Main judgment criteria
  - PT increasing

- Secondary criteria
  - blood requirement in hospital
  - survival
Perspectives

- FLYP will be used in civilian area
  - In pre-hospital settings at the end of this year.
  - In the emergency department (since 2013 in Lille)
  - In austere settings

- Lyophilization process will be proposed to other blood establishments to decrease unit cost

- Preparation from whole blood

EDQM Symposium
Plasma for direct clinical use

Novel component:
freeze-dried plasma

Christophe Martinaud (MD,PhD)
French Military Blood Institute
Clamart, France

Strasbourg, mardi 22 septembre 2015
Use of plasma in massive haemorrhage

Coagulopathy of massive bleeding

- Coagulopathy secondary to substitution with crystalloids and colloids
- Coagulopathy secondary to haemotherapy
- Coagulopathy due to hypothermia and metabolic acidosis
- Coagulopathy secondary to trauma, shock

Micro Vascular Bleeding

Transfusion packages

- For massive / life-threatening bleedings
- Stored thawed AB RhD negative plasma - immediately available for transfusion
- The package consists of 5 RBC, 5 FFP, 2 PC, resulting in a hematocrit ~30%, factor concentration >30% and a platelet count ~80 x10⁹/L
- The packages are administered consecutively until haemostasis is secured

Johansson et al. Transfusion 2007

Package approach - rAAA

- Randomised clinical study was not possible due to the inability to achieve informed consent
- Patients operated for a ruptured abdominal aortic aneurysm (rAAA).
- Prospective intervention (12 months) - retrospective control (24 months) study.
- Intervention: 2 PC at start of surgery and 2 PC during reperfusion. FFP was administered in a 1:1 ratio with RBC from start of surgery.
- Control group was transfused according to existing guidelines.

P<0.05

Control
Intervention

Johansson et al. Transfusion 2007

EDQM Symposium on "Plasma for Direct Clinical Use", 22-23 Sept. 2015
Damage control resuscitation - A meta-analysis

Johansson et al.  JETS 2012

- Search for trauma patients receiving 10 RBC or more in 24h or less in 2005-2010
- Nineteen retrospective studies evaluated and 15 studies included (N=3,475)
- Highest ratio vs. lowest FFP and/or PLT to RBC ratios evaluated

Holcomb et al. JAMA 2015

EDQM Symposium on "Plasma for Direct Clinical Use", 22-23 Sept. 2015

Transfusion of Plasma, Platelets, and Red Blood Cells in a 1:1:1 vs a 1:1:2 Ratio and Mortality in Patients With Severe Trauma
The PROPR Randomized Clinical Trial

Johansson et al. JETS 2012

Table 2. Trial Outcomes by Treatment Group
Two hundred sixty-four trauma patients

Demographics and ISS were similar between groups

Table 3. Adjudicated Cause of Death by Treatment Group and Period From Randomization

<table>
<thead>
<tr>
<th>Cause of Death</th>
<th>No. (%)</th>
<th>Difference (95% CI), %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exsanguination</td>
<td>31 (3.2)</td>
<td>50 (3.6)</td>
</tr>
<tr>
<td>Traumatic brain injury</td>
<td>11 (1.1)</td>
<td>13 (1.4)</td>
</tr>
<tr>
<td>Respiratory, pulmonary contusion, or tension pneumothorax</td>
<td>3 (0.3)</td>
<td>1 (0.1)</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Multiple organ failure</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Calculated using exact unconditional method based on the Farrington-Manning score statistic.

* A patient may have had more than 1 cause of death.
Is plasma more than just volume and coagulation factors?

More on this later

The blood bank: from provider to partner in treatment of massively bleeding patients

Pär I. Johansson

Real-Time Monitoring and Guidance

Blood bank

>10 blood products / 24 h.

>20 RBC+FFP

> 10 RBC

Patient / patient

Fig. 1. Algorithm for monitoring administration of blood products to massively bleeding patients.
Monitoring of coagulopathy

Monitoring of coagulopathy has relied on plasma-based coag. tests such as:

- PT/INR
- APTT
- Fibrinogen conc.
- D-dimer
- Platelet count

Whole blood haemostatic assays are required

Whole blood analysis
Measures the viscoelastic properties of the clot
Global assessment of clot build up and degradation
Rapid tests (15 min.)

Viscoelastical haemostatic assays (VHA)

- TEG®
- ROTEM®
VHA principle and clinical relevance

**Diagram:**
- Torsion wire
- Coagulation
- Clotting time (R)
- Fibrinolysis (LY)
- Platelet function (MA)
- Coag. factors, Fibrinogen, PLT
- Fibrinogen, FXIII, PLT
- Coag. factors and platelets (PLT)

**Graph:**
- nM thrombin
- Minutes
- Peak
- tt Peak

**Citation:** Kawasaki et al. 2004
VHA traces and coagulopathy

- Normal
- Hypocoagulable I
- Hypocoagulable II
- Hypocoagulable III

- Heparinization
- Hypercoagulable
- Reactive fibrinolysis
- Primary hyperfibrinolysis

Copenhagen Concept

Blood bank MD on call
Effect of Haemostatic Control Resuscitation on mortality in massively bleeding patients: a before and after study

P. L. Johansson and J. T. Stenballe
1Department of Clinical Immunology, Blood Bank, and 2Department of Anaesthesiology, Centre of Head and Orthopaedics, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark

Number at risk Time in days
2005-2006 442 385 362 352 347 345 344 343 343
2002-2003 390 308 281 267 257 255 255 255 255

Log-rank, \( P < 0.001 \)

How I Treat

How I treat patients with massive hemorrhage

Pål L. Johansson,1,2 Jakob Stenballe,3,4 Roberto Oveiri,5 Charles E. Wade,6 Sisse R. Ostrowski,1 and John B. Holcomb3
1Section of Transfusion Medicine, Capital Region Blood Bank, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark, 2Department of Surgery, Division of Acute Care Surgery, Centre for Translational Hope Research, University of Texas Health Medical School, Houston, TX, and 3The Trauma Centre, Department of Anaesthesiology, Centre of Head and Orthopaedics, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark

Massive hemorrhage is associated with coagulopathy and high mortality. The transfusion guidelines up to 2006 recommended that resuscitation of massive hemorrhage should occur in successive steps using crystalloids, colloids, and red blood cells (RBCs) in the early phase and plasma and platelets in the late phase. With the introduction of the coagulase model of hemorrhage in the mid-1990s, our understanding of the hemostatic process end of coagulopathy has improved. This has contributed to a change in resuscitation strategy and transfusion therapy of massive hemorrhage along with an acceptance of the adequacy of whole blood for hemorrhagic shock and not just RBCs. Thus, in 2006, a strategy aiming at avoiding coagulopathy by proactive resuscitation with blood products in a balanced ratio of RBCs/plasma/platelets was introduced, and this has been reported to be associated with reduced mortality in observational studies. Consequently, whole blood viscoelastic hemostatic assays have gained acceptance by allowing a rapid and timely identification of coagulopathy along with identifying an individualized, goal-directed transfusion therapy. These strategies joined together seem beneficial for patient outcomes, although final evidence on outcomes from randomized controlled trials is lacking. We present here how we in Copenhagen and Houston, today, manage patients with massive hemorrhage.

Table 1. TEG treatment algorithm from Copenhagen

<table>
<thead>
<tr>
<th>TEI variable</th>
<th>Normal range</th>
<th>Value</th>
<th>Comment</th>
<th>Hemostatic therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>R: (min).</td>
<td>0-10</td>
<td>10-0</td>
<td>Coagulation factor 1</td>
<td>Platelets,凝血因子1</td>
</tr>
<tr>
<td>Angle:</td>
<td>20-45</td>
<td>&gt;45</td>
<td>Coagulation factors 2,3</td>
<td>Platelets,凝血因子2,3</td>
</tr>
<tr>
<td>Reaction time:</td>
<td>2-7</td>
<td>&gt;7</td>
<td>Thrombin generation</td>
<td>Platelets</td>
</tr>
<tr>
<td>Kinetic TEG MAV:</td>
<td>20-40</td>
<td>&lt;20</td>
<td>Thrombin generation</td>
<td>Platelets,凝血因子2,3</td>
</tr>
<tr>
<td>Kinetic TEG MAV:</td>
<td>0-60</td>
<td>&gt;60</td>
<td>Thrombin generation</td>
<td>Platelets,凝血因子2,3</td>
</tr>
<tr>
<td>R in Kinetic TEG MAV:</td>
<td>0-15</td>
<td>&gt;15</td>
<td>Thrombin generation</td>
<td>Platelets,凝血因子2,3</td>
</tr>
</tbody>
</table>

Coagulation TEG-derived guidelines for hemorrhagic resuscitation with plasma, platelets, cryoprecipitate pool, fibrinogen concentrate, and tranexamic acid in bleeding patients were used at the Capital Region of Denmark. In patients with trauma to the thoracic area, MA
Acute coagulopathy of trauma: Balancing progressive catecholamine induced endothelial activation and damage by fluid phase anticoagulation

P. J. Johansson, S. R. Ostrowski
Section for Transfusion Medicine, Capital Region Blood Bank, Rigshospitalet, University of Copenhagen, Blegdamsvej 9, DK-2100 Copenhagen, Denmark

Medical Hypotheses 75 (2010) 564–567

Early shock induced coagulopathy reflects systemic endotheliopathy
Coagulopathy is a result of systemic ENDOTHELIOPATHY and this predicts trauma patient outcome

**Disrupted vascular integrity**

**What Happens to the Body’s Organs?**
**A Vicious Cycle**

- Fluid resuscitation for critical illness
- Brain: Increased intracranial pressure
- Lungs: Edema, hypoxemia, hypoxia
- Intestines: Decreased blood flow to intestines, fecal output, congestion, decreased UOP
- Head: Reduced cardiac output, false elevation in CVP and wedge
- Kidneys: Decreased blood flow to kidneys, reduced renal perfusion, decreased UOP
- Venous Cava: Compressing, resulting in reduced blood flow back to heart, reduced preload
- Total body fluid third spacing/edema
- Elevated intra-abdominal pressure due to bowel edema
- Vena cava compression
- Multi-system organ dysfunction/failure
- Reduced blood flow to organs
- Reduced cardiac output
- Reduced blood return to heart (preload)

Wataha et al. Transfusion 2013
Plasma Restoration of Endothelial Glycocalyx in a Rodent Model of Hemorrhagic Shock

A. Sham
B. Negative Control
C. Shock
D. Lactated Ringers
E. Plasma


Protective Effects of Fresh Frozen Plasma on Vascular Endothelial Permeability, Coagulation, and Resuscitation After Hemorrhagic Shock Are Time Dependent and Diminish Between Days 0 and 5 After Thaw

Shibani et al. J Trauma. 2010

p<0.05 and HS vs B
*p<0.05 FFP Day 0; FFP Day 5 vs end HS
Adiponectin in Fresh Frozen Plasma Contributes to Restoration of Vascular Barrier

Function after Hemorrhagic Shock

Xiyun Deng 1*, Yanna Cao 1*, Maria P. Huby 2, Chaojun Du 1, Lisa Baer 2, Zhanglong Peng 3, Rosemary A. Kozar 1, Marie-Francoise Doursou 1, John B. Holcomb 1,2, Charles E. Wade 1,2 and Tien C. Ko 1

Endothelial “rescue” with plasma improves outcome!!

Hemorrhagic shock is the leading cause of preventable deaths in civilian and military trauma. Use of fresh frozen plasma (FFP) in patients requiring massive transfusion is associated with improved outcomes. FFP contains significant amounts of adiponectin, which is known to have vascular homeostatic function.

Adiponectin in FFPabolished FFP’s effects on blocking endothelial hyperpermeability in vitro, and on improving lung vascular barrier function in HS mice. Replenishment with adiponectin restored FFP’s effects. These findings suggest that adiponectin is an important component in FFP rescue and contributing to the beneficial effects on vascular barrier function after HS.

Thank you!