Plasma fractionation - Variety of Products

Paul Strengers
Sanquin Plasma Products
Amsterdam
The Netherlands

IPFA / BCA  Global Symposium on “The Future for Blood and Plasma Donations,
Fort Worth
September 28, 2015

Blood and Beyond
From donor ....
…. manufacturing of plasma products…..
…. to patients.
Plasma fractionation - Variety of Products

• Production process of plasma products

• Variety of products and clinical application
Blood components and plasma products

- Red blood cells
- Platelets
- Fresh Frozen Plasma

Blood components

- Albumin
- Immunoglobulins
- Coagulation factors
- C1-inhibitor concentrate
- Other proteins

Pooling and production

Plasma products / medicines

28 September 2015
Blood plasma

- > 1,000 proteins
- about 250 proteins identified
- 117 proteins FDA licensed assays
- ~ 20 proteins commercially used as therapeutics

Dynamic range of single proteomic techniques
Total Cost: $10,695 per sample
Plasma fractionation

• Purification of therapeutic plasma proteins

• Somewhat comparable with dairy industry:
  * Protein solution as source
  * One source material contributes to multiple products
  * Hygienic importance

• Important differences with dairy industry:
  * Tight measures on transmission of blood borne infections
  * Specific regulations
  * Pharmaceutical production process
Cohn fractionation

• Differences in solubility of proteins influenced by e.g.:
  - Alcohol concentration
  - Temperature
  - pH
  - Ionic strength

⇒ Variations in process conditions lead to separation of proteins

Edwin Joseph Cohn 1892-1953
C1 esterase inhibitor
Prothrombin complex
Factor IX
Factor VIII
Plasma
Cryoprecipitate
Cryo-poor plasma
3F-eluate
Factor IX
4F-eluate
Prothrombin complex
Residual plasma
4F, C1-INH poor plasma
Cryo, 4F-poor plasma
Cryo, 3F, 4F-poor plasma
DS-eluate
C1 esterase inhibitor
Plasma fractionation scheme (1)
Plasma fractionation scheme (2)

Specific IgG’s:
- HB IgG
- VZ IgG
- T IgG
- Rh(D) IgG

Immunoglobulin (IV)

Immunoglobulin (IM/SC)

Residual plasma

Fraction I

Fraction II + III

Fraction III

Fraction I + II + III

Fraction IV

Fraction I + III

Fraction II

Fraction II

Powder II

Fraction V

Albumin

28 September 2015
Source material
The plasma arrives.....

1250 - 1600 Kg plasma per batch = ± 5000 - 6000 donates
Frozen for storage

Cold precipitation

DNA / RNA

lipid-envelop

Protein coat

solvens-detergents, bijv. TNBP and Tween

AIDS-virus (HIV)

SD treatment

Cryoprecipitate is used for factor VIII
Virus-removing treatments

- **Virus-removing treatments**
  - **Protein precipitation**
  - **SD treatment**
  - **Monoclonal purification**
  - **Filtration (nanofiltration)**
  - **Pasteurisation**

In European guidelines:
- At least 2 effective virus-reducing steps with different mechanisms for enveloped viruses.
- At least 1 effective step for non-enveloped viruses.

Log reduction $> 4 \log_{10} 10$ is defined as effective step.

$4 \log_{10} 10 \Rightarrow$ reduction factor 10,000 (99.99% reduction)
Semi-automated purification of factor VIII:

- Immuno-affinity chromatography
- Ion exchange chromatography
Paste II is the source for immunoglobulins
High-quality technologies for further manufacturing, purification, and virus-inactivation or virus-removing treatments....
Plasma fractionation – Variety of Products

• Production process plasma products

• Variety of products and clinical application
  • Clotting factors and coagulation
  • Albumin
  • Alpha-1 proteinase inhibitor
  • C1-esterase inhibitor concentrate
  • Immunoglobulins
  • Hyper-immune globulins or specific globulins
  • C1 esterase inhibitor
Variety of Products
Clotting factors and clinical usage

• **Factor VIII**
  • Hemophilia A

• **Factor IX**
  • Hemophilia B

• **Activated prothrombin complex concentrate (Anti-Inhibitor Coagulant Complex)**
  • Factor VIII inhibitor bypassing agent
  • Control of spontaneous bleeding episodes and use in surgery in hemophilia A and B patients with inhibitors

• **Prothrombin complex concentrate**
  • Factor II, VII, IX and X, or without VII
  • Reversal VKA treatment or congenital deficiency of factor

• **Antithrombin III**
  • Inherited deficiency of anti-thrombin III
Variety of Products
Clotting factors and coagulation

• **Protein C**
  • Severe congenital Protein C deficiency, for the prevention and treatment of venous thrombosis, and purpura fulminans

• **Factor XIII**
  • Congenital deficiency of Factor XIII and resultant haemorrhagic diathesis, haemorrhages and disturbances in wound healing.

• **Factor VII, factor XI, factor XIII**
  • These plasma derived clotting factor concentrates are used to treat patients with deficiency of the relevant clotting factor

• **von Willebrand factor (VWF)**
  • Von Willebrand’s disease

• **Fibrinogen (Factor I)**
  • Congenital fibrinogen deficiency (eg, afibrinogenemia, hypofibrinogenemia)

• **Fibrin Sealant**
  • Creating fibrin clot
Variety of Products

- **Albumin**
  - Restoration and maintenance of circulating blood volume where volume deficiency has been demonstrated

- **Alpha-1 proteinase inhibitor**
  - Alpha-1 antitrypsin deficiency

- **C1-esterase inhibitor concentrate**
  - Hereditary angioedema

- **Immunoglobulins**
  - Multiple indications

- **Hyper- immunoglobulins or specific immunoglobulins**
  - Passive immunization
Indications of immunoglobulins

Substitution
- Primary immune deficiency
- Secondary immune deficiency

Immune modulation
- Idiopathic Thrombocytopenic Purpura (ITP)
- Guillain-Barré syndrome
- Kawasaki’s disease
Off-label indications of IVIG

- Abortion (spontaneous)
- M. Alzheimer
- Asthma
- Autism
- Chron. Inflam. Demyel. Polyneuropathy (CIDP)
- Critical Illness polyneuropathy (CIP)
- M. Crohn
- Dermatomyositis / polymyositis
- Diabetes mellitus
- Hematological coagulation disorders
- Hematological, immunological cellular disorders
- Infections in neonates
- Multiple Sclerosis (MS)
- Multifocal Motor Neuropathy (MMN)
- Myasthenia Gravis
- Parvo-B19 associated anemia
- Post-transfusion Purpura
- Rheumatoid arthritis
- Sepsis
- Stiff Person Syndrome
- SLE
- Therapy resist. epilepsy in children
- Etc. Etc.
Hyper-immune globulins

• Plasma from immunized and/or reconvalvescent donors

• Post-exposure prophylaxis
  - Hepatitis B immunoglobulin
  - Varicella zoster immunoglobulin
  - Tetanus immunoglobulin
  - Rabies immunoglobulin
  - Pertussis immunoglobulin
  - Cytomegalovirus immunoglobulin
  - Rhesus (D) immunoglobulin
  - (anti- Ebola, anti- Echo virus, anti-diphteria, anti-measles, anti-pneumococcus, anti-rubella, anti-polio, anti-respiratory syncytium virus, anti-vaccinia, etc.)
Rh(D) immunoglobulin

• Rh(D) negative woman and Rh(D) positive infant
  • Anaemia and fetal hydrops
  • Severe haemolytic disease of the newborn (HDN)
    • Oedema, hepatosplenomegaly, severe anaemia severe jaundice and/or death
• Rh(D) immunoglobulin at 30 weeks and at birth (within 48 hours)
C1-esterase inhibitor concentrate

- Since 1972 available in Europe
- Since 2008 available in the USA
- Treatment and/or prophylactic use in HAE
- Increased usage in Europe and USA
- Home treatment
C1-esterase inhibitor concentrate for hereditary angioedema

- The disease is congenital
- Unexpected swellings
- Painful and sometimes very dangerous
# Novel Plasma Proteins under Development

<table>
<thead>
<tr>
<th>Protein</th>
<th>Isolation (Cohn Fractionation)</th>
<th>Disease 1</th>
<th>Disease 1 Incidence</th>
<th>Disease 2</th>
<th>Disease 2 Incidence</th>
<th>Disease 3</th>
<th>Disease 3 Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transferrin/Apotransferrin</td>
<td>Fraction IV-4</td>
<td>Congenital deficiency</td>
<td>Worldwide - 10-20 patients</td>
<td>β Thalassemia</td>
<td>US Prevalence is 1,000 patients</td>
<td>Sickle Cell Anemia</td>
<td>US Prevalence is 70,000-90,000 patients</td>
</tr>
<tr>
<td>Ceruloplasmin</td>
<td>Fraction IV-1</td>
<td>Congenital deficiency (acerotoplasmminemi a)</td>
<td>US Prevalence is 315 patients , or 1:1M population</td>
<td>Wilson Disease</td>
<td>US Incidence is 3,000-12,000 patients , or 1-4:100k population.</td>
<td>Menke Disease</td>
<td>US prevalence is 1,200 - 2,700 patients.</td>
</tr>
<tr>
<td>Factor H</td>
<td>Fraction II+III</td>
<td>aHUS (atypical Hemolytic-uremic syndrome)</td>
<td>US Incidence is 15,000-60,000 patients</td>
<td>Dry Age-Related Macular Degeneration</td>
<td>US Prevalence is 12,700,000 patients</td>
<td>Wet Age-Related Macular Degeneration</td>
<td>US Prevalence is 1,600,000 patients</td>
</tr>
<tr>
<td>Factor V</td>
<td>None - new process</td>
<td>Congenital Factor V Deficiency</td>
<td>US Prevalence is 163 patients.</td>
<td>Congenital Factor V/Factor VIII Deficiency</td>
<td>US Prevalence is ~100-300 patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibronectin</td>
<td>Cryoprecipitate</td>
<td>Wound Healing</td>
<td>Cancer (leukemia)</td>
<td>Coronary artery disease in type 1 diabetes</td>
<td>US Prevalence is 25,000-50,000 patients for Type 1 Diabetes</td>
<td>Crohn’s disease</td>
<td>North America prevalence is 400,000-600,000 patients</td>
</tr>
<tr>
<td>Haptoglobin</td>
<td>Fraction IV-1, IV-4</td>
<td>Diabetic Neuropathy</td>
<td>Acute coronary syndrome (ACS) is 1.255M new or recurrent coronary attacks annually, and 450,000 deaths.</td>
<td>Congenital HDL deficiency (Tangier disease)</td>
<td>Worldwide - about 50 patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Density Lipoproteins A</td>
<td>Fraction IV-1</td>
<td>Selective IgA deficiency</td>
<td>US Prevalence is 500,000-1,000,000 patients</td>
<td>Celiac disease with IgA deficiency</td>
<td>US Prevalence is 4,000 patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA</td>
<td>Fraction II+III</td>
<td>Acute peripheral arterial occlusion (aPAO)</td>
<td>US Incidence is 100,000 patients</td>
<td>Ischemic stroke</td>
<td>US Incidence is 360,000 patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasmin</td>
<td>Fraction II+III</td>
<td>Ligneous Conjunctivitis</td>
<td>US Prevalence is 210 patients, or about 1.5:1M population.</td>
<td></td>
<td>US Incidence is 3,600-7,200 patients with Ischemic Stroke &amp; DVT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein S</td>
<td>Fraction IV-1</td>
<td>Congenital Protein S Deficiency</td>
<td>US Prevalence about 100,000 patients</td>
<td>Acquired Protein S Deficiency</td>
<td>US Incidence is 3,600-7,200 patients with Ischemic Stroke &amp; DVT</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Summary

• One blood- or plasma-donation may contribute to many plasma-derived medicinal products
• Plasma products have an important place in the treatment of many patients
• Plasma products have multiple functions in clinical therapy and these functions will be further developed
• Care will become more patient-driven (e.g. more concentrated products, patient friendly ways of administrations)
• New plasma products will be developed for ‘niche markets’

• The need for sufficient supply of plasma will equally row