

From the Department of Molecular Medicine and Surgery  
Karolinska Institutet, Stockholm, Sweden

# **EXTRACORPOREAL MEMBRANE OXYGENATION IN TRAUMA PATIENTS WITH HYPOVOLAEMIC SHOCK**

Magnus Larsson



**Karolinska  
Institutet**

Stockholm December 4<sup>th</sup> 2015

**Cover:** SEM of oxygenator capillaries with fibrin clots and cells, Kjell Hultenby.

**Back cover:** Hat worn by Karl XI at the Battle of Lund, December 4, 1676,

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Extracorporeal Membrane Oxygenation in trauma patients with hypovolaemic shock

## THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

**Magnus Larsson, M.D.**

*Principal Supervisor:*

Professor Thomas Renné  
Karolinska Institutet  
Department of Molecular Medicine and Surgery

*Opponent:*

Professor Matthias Arlt  
University of Regensburg  
Department of Anesthesiology

*Co-supervisors:*

Professor Björn Frenckner  
Karolinska Institutet  
Department of Women and Child Health

*Examination Board:*

Professor Tor Hervig  
University of Bergen  
Department of Immunology

Ass. Professor Jan Hultman  
University of Uppsala  
Department of Surgical Sciences

Professor Claes Juhlin  
University of Uppsala  
Department of Surgical Sciences

Ass. Professor Michael Broomé  
Karolinska Institutet  
Department of Physiology and Pharmacology

Professor Jan Van der Lindén  
Karolinska Institutet  
Department of Molecular Medicine and Surgery



“Contra vim mortis non est medicamen”\*

- Sigismund III Vasa on his deathbed 1632

*To nine thousand soldiers,  
slaughtered on the fields  
of Lund December 4<sup>th</sup> 1676*

\*" There is no medicine against the lethal power of dea



## FÖRORD

Den här avhandlingen har varit min trogna följeslagare under de senaste fyra åren. Den är resultatet av många roliga och lärorika diskussioner med handledare och forskningskollegor, åtskilliga möten, hundratals timmar i djurlaboratoriet och på forskningslaboratoriet, tjugo högskolepoäng med tillhörande tentamina, samt inte minst de senaste sex veckornas intensiva läsande och skrivande. Boken har följt med mig, såväl på semesterresor, som på ECMO uppdrag i norra Finland. Utan mina fantastiska handledare, Thomas, Björn, Janne och Micke så hade den aldrig blivit av. Många andra vänliga människor har också bidragit. Bokens och min relation har inte varit helt okomplicerad. Det har varit en väldigt roligt men också bitvis utmanande tid. Det har nu blivit dags att lämna min första och kanske enda bok vidare. Det är inte utan viss stolthet som jag färdig förklarar min avhandling och jag hoppas att den kommer att intressera dig.

Som när ett älskat barn flyttar hemifrån. Svårt men skönt.

Så är det med den här boken också.

Trevlig läsning,

M





# ABSTRACT

## Lifesaving or Life-threatening?

If the worst thing happens, and you get seriously injured in an accident, your body has a pronounced ability to immediately stop bleedings and start the process of healing. This unique capacity only works to a certain degree. Our body cannot by itself handle life-threatening bleedings from the heart, large blood vessels or severe injuries to highly vascularized organs. Transfusion of blood products, acute surgery and intensive care are necessary to support the body. In devastating injuries even this may not be enough and new ways of treating massive bleeding needs to be explored. Sometimes our body's rescue system gets overloaded and starts to counteract the intended positive reaction. Excessive coagulation with a resulting occlusion of a blood vessel is one example. In that case anticoagulation is needed. Another example is if inflammation, the process necessary to start healing, derails. This may cause an overwhelming inflammation in the lungs that abolish their ability of saturating our blood. If the lungs take time off, a heart lung machine can buy time for the healing and save the life. A heart lung machine (ECMO) needs anticoagulation since the plastic tubings can cause clotting. Anticoagulation is associated with the risk of dangerous bleeding. If the system clots, and the machine shut down, a life saving procedure can suddenly change to a life-threatening situation.

This Thesis highlights both sides of the hemostatic and anticoagulative coin. We have attacked the mentioned challenges from four different angles.

In **Article I** venoarterial ECMO's effect on central blood pressure was investigated. The reason for this is that we had found an unexpected effect of ECMO, during trauma resuscitation. A young girl's severe liver bleeding suddenly could be controlled when ECMO was initiated because of lung failure. Swine were used in the study and we found that VA ECMO reduced the central venous pressure while mean arterial pressure was improved.

In **Article II** a novel way of anticoagulation or "thromboprotection" in an ECMO system was evaluated. 3F7, an antibody that inhibits the activated form of coagulation factor XII was studied on rabbits connected to ECMO. 3F7 could prevent clotting in the ECMO system as effective as heparin but did not impair the hemostatic capacity and did not increase wound bleedings.

In **Article III** venoarterial ECMO's effect on rabbits in lethal traumatic bleeding shock was evaluated. Focus was on how the ECMO treatment affects central circulation, temperature, acid-base balance and the coagulation ability. ECMO efficiently increased the temperature, stabilized the circulation, improved the pH and ameliorated the hemostatic capacity.

In **Article IV** Polyphosphate (PolyP), a substance that is released from activated platelets and that induces coagulation by activating FXII was investigated. Liver injuries in swine were treated with PolyP, Kaolin and a non-active substance. PolyP efficiently initiated thrombin formation and terminated bleeding as efficiently as Kaolin but with less inflammation.

## LIST OF SCIENTIFIC ARTICLES

The thesis is based on the following articles, which are referred to in the text by their Roman numerals:

- I. **Larsson M**, Talving P, Palmér K, Frenckner B, Riddez L, Broomé M.  
Experimental extracorporeal membrane oxygenation reduces central venous pressure: an adjunct to control of venous hemorrhage?  
*Perfusion* 2010 25(4) 217–223
  
- II. **Larsson M**, Rayzman V, Nolte M, Nickel K, Björkqvist J, Jämsä A, Hardy M, Fries M, Schmidbauer S, Hedenqvist P, Broomé M, Pragst I, Dickneite G, Wilson M, Nash A, Panousis C, Renné T.  
A Factor XIIa Inhibitory Antibody Provides Thromboprotection in Extracorporeal Circulation Without Increasing Bleeding Risk  
*Sci Transl Med* 2014 Vol 6 Issue 222 222ra17
  
- III. **Larsson M**, Forsman P, Hedenqvist P, Östlund A, Hultman J, Wikman A, Riddez L, Frenckner B, Wahlgren C-M.  
Extracorporeal Membrane Oxygenation Improves Coagulopathy in an Experimental Traumatic Hemorrhagic Model  
*Submitted Manuscript*
  
- IV. **Larsson M**, Österholm C, Hultenby K, Frenckner B, Hultman J, Renné T.  
Polyphosphates are Hemostatic Agents In Vivo  
*Manuscript*

## POPULÄRVETENSKAPLIG SAMMANFATTNING

Kroppsskador i samband med olyckor och våld är ett allvarligt problem, inte bara för den enskilda individen och dennes anhöriga, utan även för samhället. Det är huvudsakligen unga människor i arbetsför ålder som drabbas av svåra skador och i åldern 5-45 år är trauma den vanligaste dödsorsaken. Förutom den svåra psykosociala påfrestningen, det innebär för drabbade familjer, så uppgår kostnaderna för samhället till miljardbelopp för vård och förlorad arbetskraft. Under de senaste decennierna har omhändertagandet av svårt skadade patienter förbättrats avsevärt. Genom Advanced Trauma Life Support (ATLS) kurserna, som initierades i USA i början av 1980-talet och som successivt förbättrats och spridits internationellt, har det initiala omhändertagandet blivit allt mer strukturerat. Den traumakirurgiska kompetensen på sjukhus runt om i världen har också stärkts genom Damage Control Surgery- och Definitive Trauma Surgical Care utbildningar. Det har blivit alltmer uppenbart att den svårt skadade patienten, efter akut åtgärd av livshotande luftvägs- och blödningsproblem, behöver en normaliserad fysiologi snarare än en perfekt återställd anatomi. Kunskaper och erfarenheter vunna från krigs- och katastrofområden, med många allvarligt skadade under kort tid, implementeras i den civila sjukvården och civila tekniker och utrustningar flyttas närmare stridsfältet. En ledstjärna i modern traumavård är att inte åstadkomma mer skada än nödvändigt vid en kirurgisk behandling, s.k. minimal-invasiv kirurgi. Detta för att den redan skadade kroppen inte ska belastas ytterligare. Ett bra exempel på det är blodkärlsröntgen, s.k. angiografi, där blödningar i olika organ kan stillas på ett enkelt och skonsamt sätt. Trots stora förbättringar av traumavården dör fortfarande alltför många människor efter trauma och nya obeprövade behandlingssätt behöver identifieras och utvärderas.

Extracorporeal Membranoxygenation (ECMO) är en behandling som vidareutvecklats från hjärt-lung maskin behandling (CPB) och som sedan början av 1980-talet används för att stödja hjärt- och lungfunktion på intensivvårdspatienter där konventionell behandling inte är tillräcklig. ECMO är traditionellt inte använt vid behandling av skadade personer även om sporadiska fallrapporter där behandlingen använts i det akuta skedet till döende patienter efter trauma förekommer. Det finns dock endast en tidigare känd djurstudie där ECMOs effekt vid blödningschock undersökts och då utvärderades effekten av djup nedkylning. Vissa fördelar med ECMO vid trauma är logiska och enkla att förstå. Både hjärt- och lungfunktionen kan stödjas på ett säkert sätt. De grova kanylerna tillåter snabb ersättning av stora mängder blodprodukter, blodet kan värmas, syresättas och renas från koldioxid. Kroppsorgan, som t.ex. levern och lungorna, kan kopplas ur cirkulationen vilket medger reparation av dessa organ utan att patienten blöder ihjäl och med bibehållen systemcirkulation.

I det här doktorsavhandlingsarbetet har vi undersökt vilka för- och nackdelar ECMO-behandling kan ha i det allra mest akuta omhändertagandet av skadade. Vi har valt att arbeta med gris- och kanin modeller på grund av att de är väldigt lika människor både anatomiskt och fysiologiskt. Dessa djurs koagulationssystem är också nästan identiska med människans. Det är mycket viktigt att poängtera att alla djur har varit djupt sövda, inte har kunnat uppleva försöken och inte har känt någon smärta. Under alla försök har specialutbildade djurskötare kontrollerat djuren och i alla kaninförsök har en veterinär deltagit.

I korthet har vi undersökt vad som händer med blodtrycken i kroppens centrala blodkärl och blodets koagulationsförmåga vid ECMO-behandling. Det visade sig att det centrala venblodtrycket sänktes signifikant samtidigt som artärblodtrycket förbättrades. Detta kan ha en positiv betydelse i samband med svåra skador och underlätta kontroll av blödningar som är svåra att hantera kirurgiskt. I en annan studie utsattes sövda kaniner för lårbensbrott och blödde 45 % av blodvolymen. ECMO-behandlingen höjde effektivt kroppstemperaturen, förbättrade den centrala cirkulationen, minskade blodets surhet och förbättrade koagulationsförmågan. Vidare undersöktes ett helt nytt sätt att blockera blodets levringsförmåga utan att öka blödningsrisken. Detta har inte tidigare lyckats med några andra kända läkemedel. Det gjordes genom att koagulationsfaktor XII (FXII) blockerades med en antikropp (3F7). Resultatet blev ett effektivt skydd mot blodpropps-bildning i ECMO-systemet och med bibehållen koagulationsförmåga. Eftersom konceptet att blockera FXII systemiskt fungerade väl, så blev nästa steg att istället aktivera FXII lokalt. Det gjordes för att uppnå den motsatta effekten, det vill säga stoppa blödning, i leverskador på gris. Polyphosphat, en substans som normalt finns i kroppens blodplättar, de blodkroppar som deltar i koagulationen, användes. Polyphosphat består av linjära molekyllängder av fosfatjoner ( $\text{PO}_4^{2-}$ ). De är kraftigt negativt laddade molekyler som aktiverar FXII. Leverblödningarna upphörde effektivt och polyphosphat orsakade ingen nämnvärd inflammation. Dessutom bröts det ned av kroppsegna enzymer och var efter 4 dygn inte längre påvisbart i blodet.

De viktigaste slutsatserna av studierna är:

- I. ECMO sänker det centrala venblodtrycket samt bibehåller eller förbättrar artärblodtrycket.
- II. Blockering av FXIIa förhindrar på ett säkert sätt koagulering i ECMO-system utan ökad blödningsrisk.
- III. ECMO förbättrar koagulationsförmågan efter livshotande blödningschock.
- IV. Polyphosphat skapar effektiv blodstillning i leverskador.

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# 1. INTRODUCTION

## History of Trauma Surgery

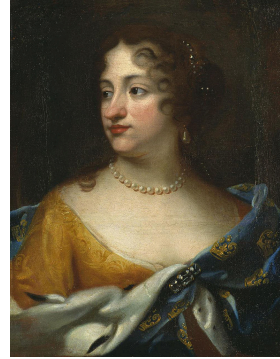
### Karl the XI <sup>1,2</sup>



Karl X Gustav (1622-1660)



Karl XI (1655-1697)



Ulrika Eleonora (1656-1693)



Lorentz Creutz (1615-1676)

Karl XI was born on November 24, 1655 at the Royal Castle, Tre Kronor in Stockholm. Karl was crowned as Sweden's King 1660, only 4 years old, at the death of his father, Karl X Gustav. Doctor Emporagrius prescribed bloodletting to the King and the nature of the blood told him that his Majesty was in a very bad condition. In the night on the 13<sup>th</sup> of February, between one and two, Karl X Gustav expired of pneumonia and sepsis. The little boy was left with a country at its peak. Sweden was after France the second largest and richest country in Europe. Sweden had conquered several landscapes from Denmark in 1658, confirmed by the Roskilde peace treaty, and the commanders were rewarded with land. Magnificent castles were built (e.g. Läckö by Magnus Gabriel De la Gardie, Skokloster by Karl Gustav Wrangel and Drottningholm by the widow Queen Hedvig Eleonora). A guardian regency administered the country until the parliament declared that Karl at 17 was of age to control the nation. Great confidence was put on the young King and he was to be taught Latin, French, German, History, Astronomy, Geography, Art and the Art of War. If Louis the XIV of France was the "Sun King", Karl was to become the "North Star". To the regency's disappointment Karl was dyslexic, had writing difficulties, and a fragile health so the Queen favored riding and hunting for him instead of theoretical studies. Karl preferred to spend time with his family and close friends at a small mansion in Kungsör. The Swedish regency had tied a close relationship with France but had ongoing conflicts with Denmark, Holland, Brandenburg and Poland. Karl tried to reach a neutrality covenant with the Danish King, Christian V, but was dismissed. The diplomat Niels Brahe was sent to Copenhagen in June 1675 with a portrait of the King and proposed on Karl's behalf to Ulrika Eleonora (Christian's sister). Ulrika Eleonora instantly fell in love and Christian initially agreed, but during the coming war he changed his mind. At that time the betrothal had already concluded and engagement rings were exchanged. Ulrika refused to break the engagement, and she even pawned her own engagement ring to be able to buy medicine and food for Swedish prisoners. In Sweden, the Lutheran Church held witch trials and more than 300 innocent young women were executed and burned at the stake. False testimonies of witchcraft often came from young children. Governor Lorentz Creutz was the chairman in the sorcery tribunal "Trolldomskommissionen" in Mora 1669 and sentenced more than 20 women to death. There was horror in the country and a growing threat from abroad.



## The Battle of Lund<sup>3,4</sup>



Karl XI at the Battle of Lund 4:th Dec 1676 (J. F. Lemke)



Karl XI (D. K. Ehrenstrahl)



Christian V of Denmark (Unknown artist)

In September 1675 King Christian V proclaimed war against Sweden just three months after his sister's engagement with Karl. Denmark wanted back the rich eastern provinces of Skåne, Blekinge, Halland, Bohuslän, Jämtland, Härjedalen and Gotland. The Swedish Navy sailed in May 1676 under command of the new Admiral Creutz, the same Creutz who had sent innocent young women to death seven years earlier. He was well known in the parliament but had no experience of the sea. Creutz led the armada on "Kronan", the largest warship ever built in Sweden (twice the size of the Royal Ship Wasa). Kronan carried 850 men and was armed with 125 bronze canons. Another 34 large warships followed her. The Navy's task was simple: "find and wipe out the Danish Navy". On June 1<sup>st</sup> 1676 they met the Danish and Dutch fleets outside the south cape of Öland. It all became a disaster. Admiral Creutz ordered a sharp turn with open gun slots, water flooded in, Kronan had a list and an ignition fell into the powder storage and the whole ship exploded. It must have been an unthinkable but glorious moment for the Danish admiral to watch the Swedish crown Jewel, one of the biggest warships in the world, explode without a single shot being fired. Maybe it was also a bittersweet revenge for the relatives of the young executed women when Commander Creutz with all his men drowned. With one exception all the other Swedish ships fled north. Several ships sank and 3000 dead sailors remained in the sea outside Öland. Now it was easy for the Danish troops to invade the island and then the mainland. Christian disembarked an army of 15000 men south of Helsingborg on June 29<sup>th</sup>. King Karl was furious when he realized what had happened and put all his advisors aside and took command of the National Defense himself. New regiments were set up and equipped in each county. This was the foundation of the Karolinska Army. Late in the fall of 1676 the Danish Army had regained Gotland, the major part of Skåne, Halland and Bohuslän and the Swedish troops were retreated to Småland. The guerilla of Salad "Snapphanarna" was educated by the Danish army to fight behind the lines and cut the enemies support. The Danes burned villages and farms. The Swedes were starving and many soldiers were in a critical condition. In November Karl reached the river Kävlingeån. All the bridges were destroyed, the shores were flooded due to heavy rains and the Danish Army was impossible to reach, located just a few kilometers south at Svenstorp's Castle. Was Karl to lose Skåne to Denmark forever? After two weeks of rain Karl was suddenly saved by the cold winter. Kävlingeån froze and field marshal Erik Dahlberg assessed the frozen ice to be strong enough to carry the heavy armed horses and artillery. Karl had to choose between Victory and Death.

He wrote to his mother that, if killed, he wanted to be buried in the Riddarholmen's Church together with his ancestors and at midnight the 4<sup>th</sup> of December he confessed to Archbishop Haquin Spegel. Three in the morning, just after the moon had disappeared, the King and the Swedish army with 8000 soldiers crossed the frozen river. The Danish troops had been partying the night before and did not react until seven in the morning when the Swedes were a few miles away. 13000 drowsy and hangover Danish soldiers tried to organize. Karl realized that the battle was better fought near the town of Lund so he started to move the troops there. Meanwhile Christian also moved his troops in parallel. All were racing towards Lund. The Swedes won the race and took place first. The battle began at 9 am. Karl's horse was shot in the head but he got the white horse Brilliant (a gift from the French King) instead. The Danish Supreme commander was injured early and together with King Christian they fled north followed by King Karl and his troops. They were followed up to Kävlingån but now the ice had thawed. Christian and his son Prince George landed safely on the other side but hundreds of Danish soldiers drowned. Meanwhile the Karoliner Army was badly beaten by the Danes at Lund. Karl should not have left his troops. Erik Dahlberg rode up to the King and convinced him to come back to Lund. When the Danish saw the King coming back they thought he was the vanguard to troop reinforcement and that they were attacked from two sides simultaneously. The right wing with 5 squadrons from Livregementets Husarer and the third cavalry regiment (today K3), was led by Colonel Nils Bielke and they were particularly successful. The Danes were shattered, fled and the final battle became a massacre of Danish and Dutch soldiers outside Vallkärra Church. The Surviving Danish troopers fled to the Landskrona Church where they were safe. For more than 18 hours the King and his men had been at horse fighting without food or water. When the night fell the victorious King Karl knelt down and accompanied by a thousand death cries from men and horses, Archbishop Spegel held a speech; "Skåden de många tusende, som på fälten runt omkring eder ligga kalla och stelnade i den eviga sömnen! Hören från när och fjärran jammerskriet av de många tusenden, som likaledes runt omkring oss ligga sargade i plågor och dödsqual. Att icke I liggen i detta ögonblick på samma sätt kvidande i dödsångesten, eller stelnade i döden, det är icke eder förtjänst, icke edert mod, icke eder skicklighet. Det är Guds hjälp, som hjälpt eder, hans nåd och barmhärtighet som förbarmat sig över eder och från edra huvuden avvänt de många tusende hotande faror."<sup>5</sup> This was one of the bloodiest days in war history. More than 9000 soldiers from different countries were killed on a single day. More casualties, than during D-Day in World War II. The 21-year-old King would never forget this day. He had overcome his timidity and taken command of the confused Swedish soldiers and extinguished the Danish Army. He would never forget the blast waves of grenades exploding, the vision of dying friends and the sound of injured horses. This was the turning point of the war and Skåne was never to become Danish again. This was the baptism of fire for the Karoliner Army and it was together with other wars around the world the birth of modern trauma surgery.

*This Thesis is dedicated to the thousands of soldiers killed on the 4<sup>th</sup> of December 1676*

## Swedish surgeons, Uppsala University and Karolinska Institutet <sup>6,7</sup>



François Du Rietz (1607-1682)



Olof Rudbeck (1630-1702)



Olof af Acrel (1717-1806)



Karl XIII (1748-1818)

### *Swedish surgeons*

The barber-surgeons were the first surgeons. They were barbers mainly from Germany and combined cutting hair and beard with bloodletting, tooth extraction and emptying of abscesses. In war times they were recruited to the army and in Sweden called "fältskärerna". Queen Christina's livemedicus barber-surgeon Balthasar Salinus came from Danzig in 1611. He was the first man to perform documented surgery in Sweden. The barbers at the Royal Court were to become responsible for the military healthcare and in 1613 it was decided that each regiment should have one barber-surgeon and four journeymen "gesäller". In Germany the titles *barbiere* und *bardscherer* were changed to "Chirurgen" and from 1647 Salinus was called Royal Surgeon "Kunglig Kirurg". Salinus' both sons Carl and Magnus followed their father and both worked for Karl XI. At the Battle of Lund brave soldiers were forced to meet the enemy eye-to-eye with guns, sabres and knives. They knew that if seriously injured the chance of survival was almost none and there were only five men with "medical training" per Regiment (1200 soldiers). With knives, scissors, branding irons, saws and trepanation-drills, injured soldiers were operated with only aquavit as the sedative. Gunshot wounds could be treated with amputations if located in an arm or a leg. Boiling oil was poured on the amputation wound to stop the bleeding. Penetrating injuries to the thorax and abdomen had to be left untreated. This commonly resulted in empyemas and peritonitis, meaning a torturous death. To spare dying soldiers suffering, mercy killings were common. Greatest chance for survival had soldiers with shallow wounds, but only if the wounds were cleaned and tetanus, gas gangrene and wound diptheria could be avoided.

### *Uppsala University*

Uppsala University, the oldest one in the Nordic countries, was founded as early as 1477. The Medical education was delayed almost 200 years. In 1656 there were 1294 students of whom only 1 (sic!) studied medicine and in 1663 there were only 12 doctors in the entire country. Olof Rudbeck, who was the first to discover the lymphatic system, defended the thesis *De Circulatione Sanguinis* in 1652. He initiated anatomy education in Uppsala and was nominated professor of medicine. In 1662 he became the principal of Uppsala University and helped the University to bloom. Olof Rudbeck was also an ancestor of Alfred Nobel, the founder of the Nobel Prize

## *Karolinska Institutet*

Grégoire François Du Rietz was the initial livmedicus (personal physician) of Karl XI. He founded "*Collegium Medicum*" 1663 to settle the physician's activities and limit quackery. Karl XI early understood the importance of trauma care and issued a Royal decree that all Swedish cities were obliged to have surgeons in 1680. Barber-surgeons had a bad reputation and to improve their knowledge and status the King initiated a test at "fältskärsämbetet" in Stockholm and Magnus Balthasar Salinus founded the Surgical Society "*Kirurgiska Societeten*" in 1686. There were constant conflicts between the academic educated Medical Physicians and the more practically educated Surgeons. Urban Hjärne who had studied medicine under Olof Rudbeck became Karl XI's livmedicus in 1684. Hjärne was also a poet and is to have said; *My son watch out for three things: for old hores, for red wine and for new doctors!* Hjärne is mainly famous for finally stopping the witch processes and execution of innocent women in Stockholm 1676. Karl XI, only 42 years old, died of a spread pancreatic cancer in April 5<sup>th</sup>, 1697. Hjärne was the most well-educated physician in Sweden, but there was nothing he could do to help his King.

In the beginning of 1700 a group of well-educated barber-surgeons came to Sweden. Olof af Acrel "*The father of Swedish Surgery*" had studied at Uppsala University and came 1735 to Stockholm to learn surgery by one of the barber-surgeons, Gerhard Boltenhagen. After his initial training he spent 5 years studying at Universities in Germany and France and got inspiration from hospitals in England and Switzerland. In 1743 he enlisted the French Army and soon became acting Chief Surgeon in Lautersburg. When he came back to Sweden he was elected a member of *Kirurgiska Societeten*. His thesis *Wound Characteristics* was the first Swedish thesis in Surgery 1745. When Stockholm's first Hospital "*Serafimerlasarettet*" was opened in 1752 Acrel became the Chief Surgeon. Acrel strongly contributed to improve the relations between surgeons and physicians and the Medical Faculty at Uppsala University awarded him a doctorate of medicine in 1760, thus making him the first *medico-chirurgus* in Sweden. Acrel who had worked unwaged at *Serafimerlasarettet* for 24 years was offered a gift of 1000 riksdaler Silver but refused to accept it. Instead he donated the money for improvements in health care. Olof af Acrel died in 1806. Four years later, Acrel's intentions were completed, when *Collegium Medicum* and *Kirurgiska Societeten* were fused. *Karolinska Institutet* was founded, by King Karl XIII, the 13<sup>th</sup> of December 13 1810, due to the high mortality in the field hospitals during the Swedish-Russian war 1808-1809. It was first named "Academy for the training of skilled army surgeons" or "*Institut för danande av skickelige fältläkare*" and was situated at the Royal Bakery on Riddarholmen, a small island in central Stockholm.

The illustration below is *Serafimerlasarettet* in 1752.



## Trauma Surgery from ancient Egypt to the 21:th Century<sup>7-9</sup>

As long as man has existed on earth, attempts to heal wounds have likely been made. It is known that the Egyptians knew how to evacuate blood from traumatic brain injuries 10000 years ago. They could also perform amputations, treat fractures, improve coagulation with raw meat and avoid wound infections with honey. During the millennia to come injuries to humans and wars around the world have forced physicians to continuously develop and improve trauma care. Medicine is probably the only field that has gained something from warfare. Below are listed important landmarks in medicine, surgery and trauma care.

### Milestones in Medicine, Surgery and Trauma Care

Year	Surgeon/Inventor	Country	Contribution
1600 BC	The Edwin Smith Papyrus	Egypt	Suture of wounds, trepanation
1550 BC	The Eber's Papyrus	Egypt	Topical treatment. Raw meat-hemostasis
1100 BC	Huang Ti	China	Anesthesia with opium and wine
600 BC	The Sushruta Samhita	India	Operations, instruments and cleanness
450 BC	Buddha	India	The first known hospital
400 BC	Hippocrates	Greece	72 Medical books, CuSO <sub>4</sub> -hemostasis
200 BC	Erasistratos	Greece	The first known human dissection
162	Galenos	Greece	Surgery for gladiators
900	Rhazes	Iraq	Cautery. Catgut suture
1170	Ruggiero Frugardi	Italy	Practica Chirurgie. Bowel suture
1363	Guy De Chauliac	France	La Grande Chirurgie
1379	John Arderne	England	Treatesis of fistula-in-ano
1460	Heinrich Von PfolSpeundt	Germany	Cauterizing GSW
1477	Jacob Ulvsson	Sweden	Uppsala University is founded
1517	Hans von Gersdorff	Germany	War surgery illustrations. Opium
1543	Andreas Vesalius	Italy	De Humani Corpori Fabrica (Anatomy)
1545	Ambroise Pare'	France	Ligature, Tourniquet
1571	Johan III	Sweden	The rights for Barber-surgeons
1628	William Harvey	England	The Heart and Blood circulation
1663	François Du Rietz	Sweden	The Swedish Collegium Medicum
1666	Richard Lower	England	Blood transfusion
1672	Richard Wiseman	England	Vascular surgery
1686	Magnus Salinus	Sweden	The Swedish Surgical Society
1741	Carl von Linne'	Sweden	Professor in Medicine Uppsala

1750	John Pringle	England	Military Medicine and Red Cross
1759	Olof af Acrel	Sweden	Chirurgiske Händelser
1765	John Morgan	USA	The first American Medical School
1783	Antoine de Lavoisier	France	Lung function
1794	John Hunter	England	Blood, Inflammation, Prim.-Sec. healing
1797-1815	Larrey (Napoleon's Surgeon)	France	Flying Ambulances. Amputations
1810	Karl XIII	Sweden	Karolinska Institutet is founded
1831	Tomas Latta	England	Saline i.v. Cholera-hypovolemia
1846	William T. G. Morton	USA	Ether anesthesia
1847	James Simpson	Scotland	Chloroform anesthesia
1847	Ignaz Semmelweis	Hungary	Chlorinated handwash
1850	Louis Pasteur	France	Bacteria. Pasteurization, vaccination
1850	Theodore Billroth	Germany	The Father of modern abdominal surgery
1855	Florence Nightingale	England	Improved standard in Military hospitals
1860	Jules-Emile Pean	France	Surgical Hemostat (Peang)
1860	Rudolph Virchow	Germany	Pathology of Hemorrhagic Shock
1867	Joseph Lister	Scotland	Antiseptic Surgery (Lancet)
1877	Robert Koch	Germany	Bacteriology, Sterilization
1894	Ludwig Rehn	Germany	First Heart Wound Repair
1895	Conrad Roentgen	Germany	X-Ray
1897	Nicholaysen	Norway	Intramedullar fixation of Femur fractures
1901	Landsteiner	Austria	Discovery of bloodgroups A, B, O
1902	Theodore Kocher	CH	International Society of Surgery
1904-1905	Princess Gedroitz	Russia	183 Laparotomies. Russian-Japan War
1916	J. McLean	USA	Heparin
1914-1918	WWI George Crile	USA	Saline i.v hemmorhagic-hypovolemia
1923	Berberich, Hirsch	Germany	Angiography
1929	Alexander Fleming	England	Penicillin
1930	John Gibbon	USA	First ECMO system
1939-1945	W W II: Blood transfusion. Antibiotics. Delayed wound closure. Faster transports to care.		
1950	Allgöwer, Mueller	Germany	AO Fracture System
1953	John Gibbon	USA	First Open Heart Surgery with CPB



1950-1953	Korean Conflict	USA-Korea	MASH. Rapid Helicopter Evacuation
1952	Frank Spencer	USA	Vascular repair, Arterial Homograft
1952	Michel De Bakey	USA	Surgery in World War II
1955-1975	Vietnam War	USA	Hemodialysis. Mechanical Vent. Blood gas
1967	Christiaan Barnard	ZA	First human-human Heart Transplant
1971	Kristensen	Germany	Ultrasound Splenic Trauma
1972	Cormack, Hounsfield	ZA, England	Computed Tomography
1972	Lauterbur, Mansfield	USA	Magnetic Resonance Imaging
1971	Hill	USA	First surviving Trauma on ECMO
1975	Bartlett	USA	First Newborn on ECMO
1980	ACS COT	USA	Advanced Trauma Life Support
1987	IATSIC: International Association for Trauma Surgery and Intensive Care		
1991	Operation Desert Storm	USA-Iraq	Intercontinental Eromedical Transfer
1993	IATSIC	ZA, CH, USA, CA, AU	Definitive Surgical Trauma Care

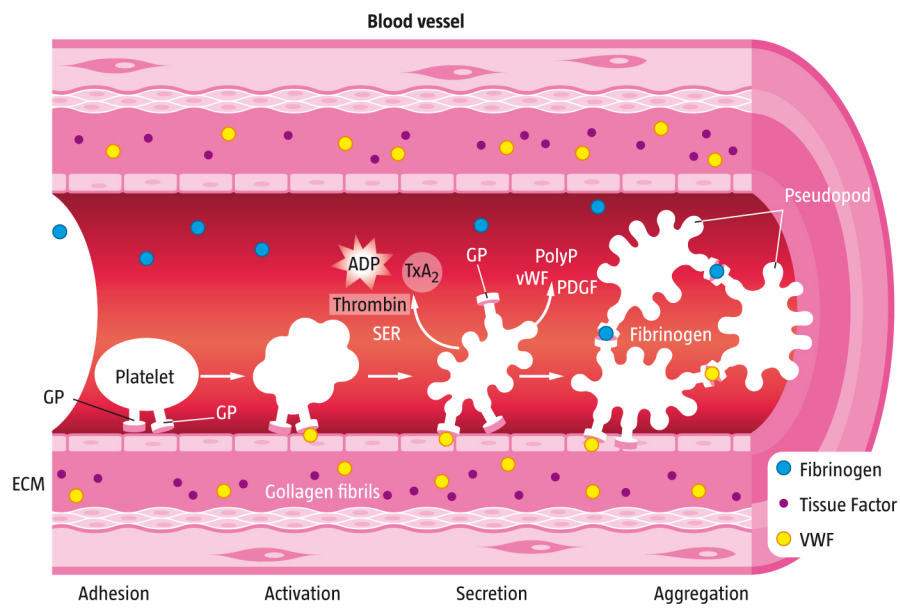
Severe injury is the leading cause of death in young adults. Trauma accounts for 4 million deaths per year (10000/day) and 1 million occur in Europe<sup>10</sup>. Injuries mainly affect people in working ages and trauma is after HIV the second most common cause of death in the 5-45-year-old group<sup>11</sup>. For every person killed in injury, several thousands of injured patients survive. Many of them live with permanent disabling sequelae. Cervical spine injuries and aortic ruptures are examples of injuries causing immediate death at the scene. Respiratory failure and bleeding shock are life-threatening complications after trauma and uncontrolled bleeding is the reason for 30% of trauma-related deaths<sup>12</sup>. Knowledge, experiences and technological advances in trauma care have been gained especially from WWI and later. Early triage and resuscitation affects outcome and trauma resuscitation has been improved during the last 20 years. The concepts and courses of ATLS<sup>13</sup>, DCS<sup>14</sup> and DSTC<sup>15</sup> described on page 35-36 all have contributed to an essential improvement in the care of severely injured patients.

## The Hemostatic System<sup>16</sup>

Humans and other vertebrates have a circulatory system with a heart, blood vessels and blood. During millions of evolutionary years nature has developed a protection- and healing mechanism for this circulatory system. The hemostatic system makes healing of injured blood vessels possible with preserved circulation and is arranged in the primary and secondary hemostasis. In mammals blood coagulation depends on more than twenty extracellular proteins, acting in a protease cascade. Every millisecond in every blood vessel, from the large aorta to the smallest capillaries, there are millions of pro-coagulant and anti-coagulant actions occurring to maintain a perfect balance of damage control and intact circulation. This balance depends upon three major players that interact with each other. The players are Platelets, Coagulation Factors and Endothelium.

### **Primary hemostasis-Platelet plug**

Platelets (PLT) are discoid enucleate cells produced in the bone marrow and that circulates in the blood. They have different surface receptors and several storage granules and are key-players for maintaining intact vascular walls and hemostasis. Immediately after an injury to a blood vessel, *vasoconstriction* and *platelet aggregation* occurs. Platelets follow a consistent pattern, *adhesion*, *activation*, *secretion* of active substances and *aggregation*. Exposed collagen and von Willebrand Factor (vWF) on the injured vessel wall lead to platelet adhesion. vWF connects the platelet to collagen through glycoprotein (GP) receptors on the platelet. Platelets aggregate to an initial thrombus, change in shape, and secrete signalling substances, Thromboxane A<sub>2</sub> (TXA<sub>2</sub>), Serotonin, Adenosine Diphosphate (ADP), Platelet Derived Growth factor (PDGF) and Polyphosphate (PolyP) from *dense bodies* and *α-granule* with vWF, FV, FXIII and fibrinogen. ADP, vWF and fibrinogen increase adhesion and aggregation of more platelets. TXA<sub>2</sub> and serotonin contract the blood vessels and reduce blood loss. PDGF increase cell proliferation in angiogenesis, the formation of new blood vessels. Prostacyclin (PGI<sub>2</sub>), Heparansulfate (HS) and Nitric Oxide (NO) from uninjured endothelium makes the vessel non-adhesive. The platelets create a binding surface upon which the coagulation cascade proceeds. Thrombin activates the platelets and Factor V, PolyP and fibrinogen accelerate coagulation (Fig. 1).

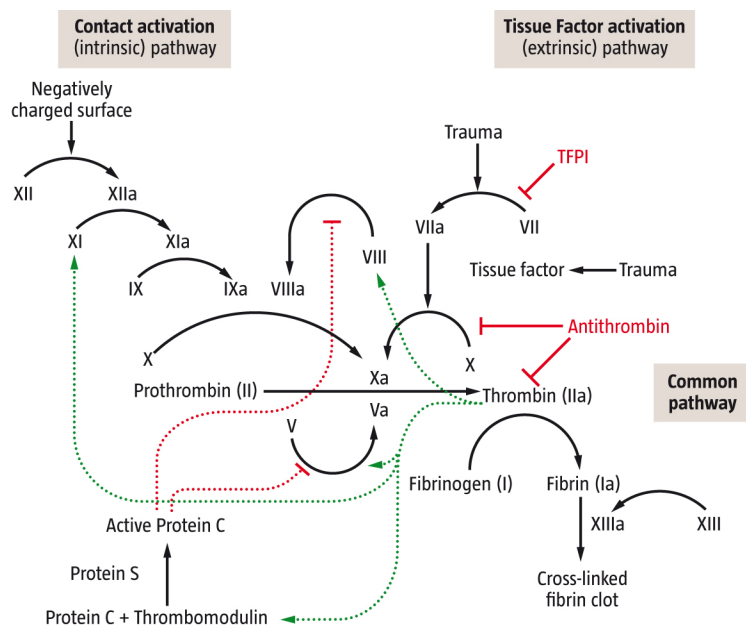


**Figure 1. The primary hemostasis-platelet plug.** Platelets contribute to hemostasis and act in four different stages. Adhesion, activation, secretion and aggregation.



## Secondary hemostasis-Coagulation

To secure and strengthen the platelet-plug, there is a need for reinforcement with *fibrin-strands* (*coagulation*). Fibrin is formed through a cascade reaction of plasma proteins, serin proteases, also called coagulation factors (F). Most coagulation factors are synthesized in the liver and circulate in plasma mainly in inactive forms (e.g. 1-2 % of FVII is active in uninjured humans). Activated thrombocytes, endothelial cells and monocytes contribute to the coagulation. Traditionally the coagulation system has been divided in the *Tissue Factor-* or *extrinsic pathway* and the *contact-* or *intrinsic pathway*. The contact pathway is initiated by contact between negatively charged ions and the zymogen FXII and is not considered to contribute to hemostasis. Coagulation in mammals is mainly initiated by FVII binding to exposed Tissue Factor (TF) on injured blood-vessel walls and the extrinsic pathway is the key to hemostasis after vessel injury. The extrinsic and intrinsic pathways leads to the common pathway where activated FX cleaves prothrombin to thrombin and thrombin in turn converts fibrinogen creating the *Fibrin clot*. In the presence of fibrin, thrombin activates FXIII and activated FXIII (FXIIIa) reinforces the fibrin clot by crosslinking the fibrin polymers. Coagulation factors acts as enzymes or cofactors.  $Ca^{2+}$  and phospholipids are important for most of their reactions. *Thrombin (FIIa)* is the central enzyme in the coagulation cascade. Thrombin activates coagulation through several coagulation factors but also induces the anticoagulant pathway through protein C, protein S and fibrinolysis. The initial formation of TF: FVIIa complex generates a small amount of thrombin. This thrombin "burst" is essential for hemostasis and feed back activates platelets, FV, VIII, X and XI, which generate more FIIa (Fig. 2).



**Figure 2. The Coagulation Cascade.** Creation of a fibrin clot (low left). The intrinsic, extrinsic and common pathways are indicated (black arrows). Thrombin's feed back activation (green). AT III, TFPI and APC inhibition (red).

## **The Endothelium**

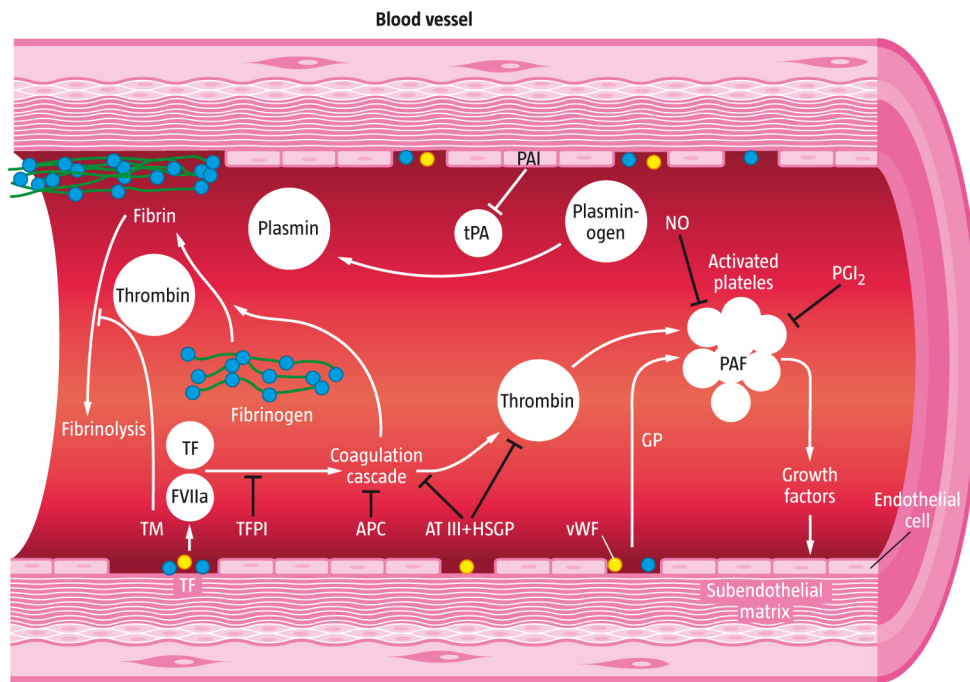
The endothelium is the inner layer of a blood vessel and it promotes blood fluidity as long as there is no injury to the vessel wall. In case of damage the endothelium promotes coagulation but prevent thrombosis. The endothelium has both pro-coagulant and anti-coagulant properties (Fig.3).

### ***Procoagulant***

If the vessel wall is injured Tissue factor (TF) a glycosylated trans-membrane protein is exposed on the surface of the vascular wall. TF binds to FVIIa and is the major activator of the extrinsic system of coagulation. The complex of TF: FVIIa activates FIX and FX that activate prothrombin (FII) to thrombin (FIIa). Endothelial cells are major producers of von Willebrand Factor (vWF), a large protein, working as a intercellular glue that binds platelets to each other and to subendothelial matrix at injury sites. vWF also act as a carrier for FVIII. vWF binds to platelet glycoprotein (GP) receptor and mediates platelet adhesion. Platelet Activating Factor (PAF) is produced both in endothelial cells and in platelets. PAF induces platelet aggregation and degranulation. The endothelium also inhibits the fibrinolytic system through activation of Thrombin activatable fibrinolytic inhibitor (TAFI) and Plasminogen activator Inhibitor (PAI). Thrombin together with thrombomodulin (TM) (endothelial cell surface receptor) activates TAFI. Activated TAFI protects the fibrin clot against lysis. PAI blocks the ability for tPA to turn on Plasmin, the most important enzyme for fibrinolysis.

### ***Anticoagulant***

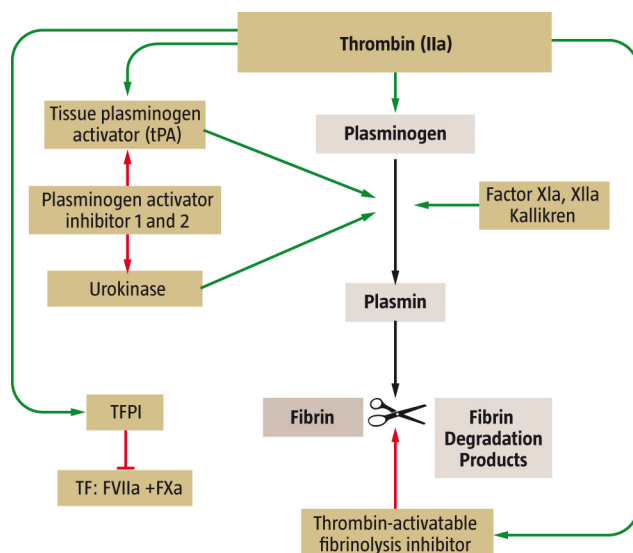
The endothelium is capable of inhibiting the coagulation pathways. Endothelial cells produces vasoactive substances and cytokines. Nitric Oxide (NO) and the prostaglandin, Prostacyclin (PGI<sub>2</sub>) both synthesized in the endothelium are powerful vasodilators and efficiently inhibit platelet aggregation. Tissue Factor Pathway Inhibitor (TFPI) inhibits the extrinsic pathway by blocking the TF: FVIIa complex. Tissue plasminogen activator (tPA) is the major activator of the fibrinolytic enzyme plasmin. When thrombin binds to thrombomodulin it can activate protein C to active protein C (APC). APC together with protein S and Ca<sup>2+</sup> proteolyze FVa and FVIIIa and this downregulates thrombin. Finally the endothelium carries Heparan-sulfate proteoglycans (HSPG) on its surface. HSPG is a cofactor for Antithrombin III (AT III), the main direct inhibitor of Thrombin, FXa, FIXa and FXIa.



**Figure 3. The Endothelium.** The endothelium has pro- and anti-coagulant properties.

## The Fibrinolytic System<sup>16</sup>

Simultaneously as coagulation occurs in response to contact activation or vessel injury the fibrinolytic pathway is activated to regulate the thrombogenic response. The fibrinolytic system is responsible for degradation of fibrin and solubilization of the formed clot. The injury in the vessel needs to be sealed but it is important that a clot does not interrupt the blood stream. Plasmin is the central enzyme in the fibrinolysis. Plasminogen is activated by thrombin, Tissue plasminogen activator (tPA), FXIa, FXIIa and Kallikrein. Plasminogen is cleaved to plasmin which degrades Fibrin to fibrin degradation products. Tissue Factor Pathway Inhibitor (TFPI) mediates an endogenous control of TF-driven coagulation. The endothelium is the major source of TFPI but TFPI is also stored in platelets and released by thrombin and other agonists. TFPI together with Protein C, Protein S and Antithrombin III (AT III) initiates the termination of pathological thrombosis. TFPI inhibits TF, FVIIa and FXa. Protein C together with the cofactor Protein S inhibits FVa and FVIIIa. ATIII is activated by vessel wall HSPG and is a very powerful endogenous anticoagulant that inhibits various proteases such as thrombin, FIXa, FXa, FXIa and the TF: FVII complex. tPA converts plasminogen to plasmin, which cuts fibrin into soluble fibrin degradation products (FDP). The fibrinolytic pathway is inhibited by Plasminogen activator inhibitor (PAI) and Thrombin activatable fibrinolysis inhibitor (TAFI). This is necessary to prevent degradation of the important hemostatic plugs (Fig. 4).



**Figure 4. The Fibrinolytic system.** Green arrows indicate activation and red arrows inhibition.

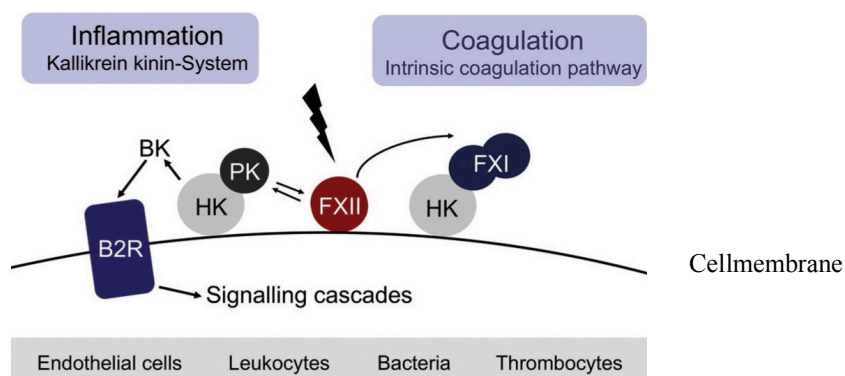


## The TF-FVIIa driven Extrinsic Pathway<sup>16</sup>

When a blood vessel gets injured the blood must rapidly clot to form a hemostatic plug to limit further blood loss. The Tissue Factor, thromboplastin, pathway of blood clotting is activated when plasma gets contact with cells that express the integral trans-membrane protein, TF, on the cell surfaces. TF binds with high affinity FVII and FVIIa, a soluble clotting factor. This causes an activation of the zymogen FVII to FVIIa. The complex of TF: VIIa adheres on the cell surface and is the most potent known activator of the blood-clotting cascade. The formed complex triggers clotting in two ways. 1. TF: FVIIa activates FIX via proteolysis. FIXa binds with a protein cofactor on a Phospholipid (PL) surface and forms the IXa: VIIIa complex. This complex catalyzes the conversion of factor X to FXa. Patients with hemophilia have a deficiency in FVIII or FIX. This makes the blood insufficient to activate FX. 2. TF: VIIa can also directly activate FX. Factor Xa together with its cofactor Va on a PL surface catalyzes the conversion of prothrombin to thrombin. Thrombin converts fibrinogen to fibrin by proteolysis. Fibrin spontaneously forms a gel. Thrombin is also a potent activator of platelets.

## The FXII-driven Plasma Contact Intrinsic Pathway<sup>16, 17</sup>

The contact system is activated by coagulation factor XII (Hageman factor) and is active on cardiovascular endothelial cells. It is a protease cascade and the plasma proteins trigger pro-coagulant reactions by the *intrinsic pathway* and pro-inflammatory reactions by kallikrein-kinin. The intrinsic pathway is initiated by FXII in a reaction that involves high molecular weight kininogen (HK) and plasma kallikrein (PK). Contact with negative ions induces a change of conformation in the zymogen FXII. This results in a small amount of active FXII (FXIIa). FXIIa cleaves plasma prekallikrein (PPK) to active PK and this activates more FXII. FXIIa initiates fibrin formation via FXI and also releases the inflammatory mediator bradykinin (BK) by PK-mediated HK cleavage. BK binds to bradykinin 2 receptors (B2R) and different pro-inflammatory signalling pathways that increase vascular permeability, dilate blood vessels and initiate chemotaxis of neutrophils and are started. The most important inhibitor of FXII and PK is C1 esterase inhibitor (C1INH) (Fig.5).



**Figure 5.** The FXII-Driven Plasma Contact System<sup>18</sup>. Courtesy of Thomas Renné.

### ***Factor XII (FXII, Hagemanfactor)***

Factor XII is a glycoprotein (single-chain) with a half-life in plasma of 50-70 hours. FXII is mainly produced in the liver and the gene is located on chromosome 5. FXII binds to negatively charged surfaces via a binding pocket in the heavy chain. FXII is activated by cleavage of a peptide bond.  $\alpha$ -kallikrein ( $\alpha$ -PK) is the most potent activator. The product of this cleavage is  $\alpha$ -FXIIa. Plasmaprekallikrein (PPK) and factor XI are the main targets of  $\alpha$ -FXIIa during contact activation and  $\alpha$ -FXIIa can be cleaved further resulting in  $\beta$ -FXIIa.  $\beta$ -FXIIa binds poorly to surfaces and does not support surface-mediated activation of PPK and FXI but activates complement factor C1 in plasma. The most important inhibitor of both  $\alpha$ - and  $\beta$ -FXIIa is C1INH but AT III and PAI also has FXIIa-blocking activity.

### ***Plasma kallikrein***

Plasma prekallikrein (PPK), Fletcher Factor is the zymogen of  $\alpha$ -PK and is primarily expressed in hepatocytes but also in pancreatic islets and kidney tubules. The primary mechanism of  $\alpha$ -PK is to cleave the high molecular weight bradykinin precursor that releases BK. PPK is converted by  $\alpha$ -FXIIa to active  $\alpha$ -PK by a proteolytic cleavage.  $\alpha$ -PK cleaves HK resulting in released BK. In a positive feed back loop  $\alpha$ -PK activates surface bound FXII to  $\alpha$ -FXIIa.  $\alpha$ -PK can also convert plasminogen to plasmin. BK initiates leukocyte migration, stimulates monocytes, induces neutrophil aggregation, chemotaxis and elastase release.

### ***High Molecular Weight Kininogen***

High Molecular Weight Kininogen (HK) is a glycoprotein predominantly expressed in the liver, but also in endothelial cells and  $\alpha$ -granules of platelets. HK is cleaved by  $\alpha$ -PK, which generates Bradykinin. HK undergoes major structural changes upon BK liberation.

### ***Bradykinin***

Bradykinin is a ligand of the B2-receptor. It is rapidly degraded to inactive peptides by angiotensin converting enzyme (ACE) and has a short half-life (< 20 s). BK is a potent local inflammatory mediator and regulates local tissue perfusion and increases vascular permeability. By initiating an increase in intracellular  $Ca^{2+}$  it activates endothelial NO formation and causes vasodilation. BK and other kinins also stimulate the production of  $PGI_2$  and  $PGE_2$ .

### ***Contact activation***

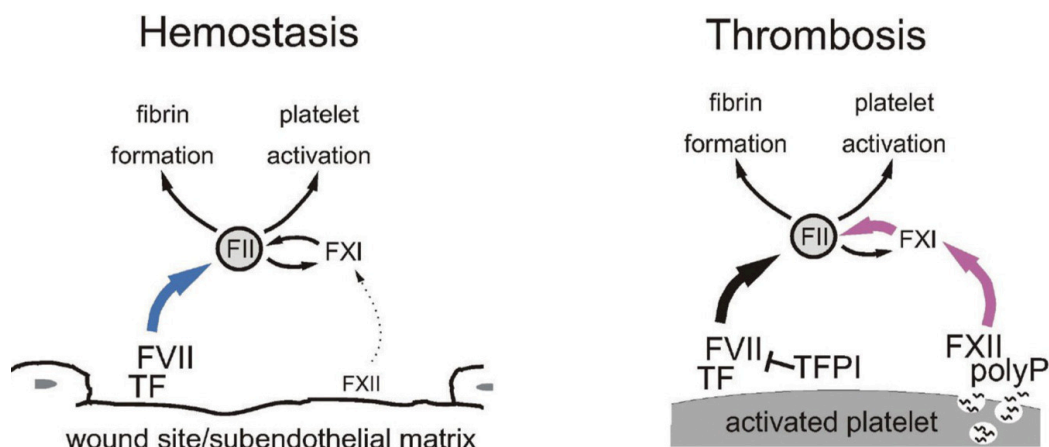
Contact activation is the mechanism of contact induced FXII zymogen activation. FXII initiates fibrin formation but has probably no function for hemostasis in humans. FXII deficient humans have no abnormal bleeding tendency despite marked prolongation in aPTT. Deficiency of the extrinsic pathway initiator, FVII results in severe bleeding. Complete TF deficiency in mice cause intrauterine bleedings and dead fetuses. Humans that lack the other contact proteins, PK or HK do not have impaired hemostasis. Similar to the FXII deficiency.

FXII can be activated by collagen in the sub-endothelial matrix, polyphosphates, and non-physiological materials like kaolin, celite, glass or certain polymers. When FXII binds to

Polyphosphates it initiates fibrin formation on procoagulant platelets with critical importance for thrombus formation.

### ***The contact system in thrombosis and hemostasis***

Fibrin formation via the intrinsic pathway is triggered by FXII and the contact system. Despite initiation of coagulation *in vitro*, patients that lack FXII have had surgical procedures with no bleeding complications. Deficiency of FXII was described the first time 1955 by Ratnoff and Colopy<sup>19</sup>. The patient John Hageman who gave name to FXII was deficient but had no increased bleeding tendency. This is in contrast to deficiencies in the extrinsic pathway (FVII, TF and FIX) and suggests that fibrin formation *in vivo* mainly is initiated by the extrinsic pathway, with TF: FVIIa. FXII-deficient mice have normal hemostasis but have deficient thrombus formation. This indicates that FXII may be crucial for pathologic thrombus formation *in vivo*. FXII gene-deficient mice are spared from the effects of cerebral ischemia in an experimental model. FXII-driven fibrin formation is specifically important for fibrin formation in thrombosis but has no function for fibrin formation during hemostasis (Fig. 6).



**Figure 6. Role of FXII in coagulation. Hemostasis:** Thrombin (FII) formation at the wound site is mainly due to TF exposed in the subendothelial matrix. TF together with FVII initiates thrombin formation leading to fibrin formation and platelet activation. FXII-contribution is minor. **Thrombosis:** When TF Pathway Inhibitor blocks FVII activity, additional fibrin forming activity is necessary to form a thrombus. Polyphosphate from platelets activates FXII and contributes to thrombin generation, platelet activation and this propagates thrombus growth. FXII and FXI deficiency, severely impairs thrombus formation, but do not deteriorate hemostasis<sup>20</sup>. Courtesy of Thomas Renné

## **Thrombosis**<sup>16</sup>

The word thrombosis originates from the Greek word thrombos, which means a clump or a clot of milk. In medicine thrombosis means the presence or formation of a blood clot. Thrombosis develops in both veins and arteries, for example deep vein thrombosis or coronary artery thrombosis. If the blood clot is detached and travels with the blood stream this is called thromboembolism and can be life threatening. This is the case when emboli end in vital organs like the lungs (pulmonary embolism (PE)), heart (acute myocardial infarction (AMI)) or brain (stroke).

### **Venous thromboembolism**

Rudolph Virchow did the first thorough analysis of the pathogenesis of thrombosis. He concluded that the consequences of the obstruction could be grouped in three categories. 1. An irritation of the vessel and its surrounding. 2. Blood coagulation disturbances. 3. The interruption of the blood stream. This combination was later named Virchow's triad. Known risk factors for venous thromboembolism (VTE) includes, increased coagulation factor activity (FV Leiden, prothrombin mutation), decreased coagulation inhibitor levels (AT-, Protein C-, and Protein S-deficiency), abnormalities in the fibrinolytic system, estrogens, pregnancy, major surgery, immobilization, active cancer, long distance travel and central venous lines. Deep Vein Thrombosis (DVT: s) most commonly occurs in the lower extremities and the most hazardous complication is an embolization to the lungs, pulmonary embolism. Low Molecular Weight Heparin (LMWH) is the most common drug both for prophylaxis and the treatment of VTE.

### **Arterial Thrombotic Disorders**

Arterial thrombotic disorders are different from VTE. They may originate in atherosclerotic plaque (AMI or stroke), in areas of low shear stress like cardio-embolism, in the arterial wall after fibro muscular degeneration leading to stenosis or aneurysms and in inflamed arteries (Takayasu's arteritis). Coronary heart disease and stroke are major causes of death in the industrialized world. Risk factors include smoking, hyperlipidemia, diabetes, obesity, and hypertension. Protective effects have been found in regularly intake of fruit and vegetables, exercise and small amounts of alcohol. Treatment of arterial thrombotic disorders varies a lot. Fibrinolytic therapy with recombinant tPA or percutaneous coronary intervention (PCI), a balloon-catheter that is inflated to open a closed coronary artery is two examples in AMI. Surgical embolectomies and bypass procedures or stents placed in blood vessels with angiographic guidance are other examples in peripheral arterial disease.

## **Therapeutic Agents targeting the Hemostatic System**

### **Anticoagulants**

Anticoagulants are drugs that prevent coagulation and clotting.

#### ***Citrate***

47% of the body's  $\text{Ca}^{2+}$  is free in solution as ionized  $\text{iCa}^{2+}$ , which is the physiologically active form.  $\text{iCa}^{2+}$  is necessary for hemostasis as a cofactor for phospholipid dependent assembly of many coagulation factors<sup>21, 22</sup>. Citrate chelates calcium and prevents activation of both platelets and the coagulation cascade. Citrate has been the standard anticoagulant for blood transfusions and stored blood products for many years.

#### ***Heparin (UNFH)***

Unfractionated Heparin (UNFH) is a glycosaminoglycan that binds to the enzyme inhibitor Antithrombin III (AT III) and increases the effect of AT III up to 1000 times. AT III effectively inhibits thrombin and FXa but also IXa, Xa and XIIa. Both the intrinsic and common pathways of coagulation are inhibited. Heparin is used as treatment of DVT, PE, as prevention for post-surgery thrombosis and to prevent clotting in ECMO-circuits, during cardiopulmonary bypass and hemodialysis<sup>23</sup>.

#### ***Low Molecular Weight Heparin (LMWH)***

Low Molecular weight Heparin (LMWH) is fractionated heparin with shorter molecule chains that causes exclusive indirect FXa inhibition. Examples are Dalteparin (Fragmin<sup>®</sup>) Tinzaparin (Innohep<sup>®</sup>) and Enoxaparin (Klexane<sup>®</sup>)<sup>24</sup>.

#### ***Direct inhibitors of FXa***

Direct inhibitors of FXa are oral anticoagulants that inhibit both the common pathway of coagulation. There is no antidote is available for these drugs. They are used as prevention of embolism in atrial flutter (AF), DVT, PE and after surgery. Two examples of direct inhibitors of FXa are Rivaroxaban (Xarelto<sup>®</sup>) and Apixaban (Eliquis<sup>®</sup>)<sup>25</sup>.

#### ***Indirect inhibitors of FXa***

An indirect inhibitor of FXa is synthetic pentasaccharide, which is chemically related to LMWH. It targets FXa rather than ATIII and inhibits FXa but not Thrombin. Fondaparinux (Arixtra<sup>®</sup>)<sup>26</sup> is one example.

#### ***Vitamin K antagonists (VKA)***

Vitamin K antagonists (VKA) blocks the liver enzyme Vitamin K epoxide reductase, that prevents vitamin K to add carboxyl groups on FII, VII, IX and X. Therefore it inhibits both the extrinsic and common pathways of coagulation. The most used VKA is the coumarin derivative Warfarin (Coumadin<sup>®</sup>, Waran<sup>®</sup>)<sup>27</sup>.

### ***Direct inhibitors of Thrombin (DTI)***

There are two types of direct inhibitors of thrombin (DTI). Univalent DTIs bind to thrombin's active site, e.g. Dabigatran (Pradaxa<sup>®</sup>). Bivalent DTIs binds both to the active site and exosite 1. and have a transient inhibition profile, e.g. Bivalirudin (Angiomax<sup>®</sup>). The anticoagulant effect of DTIs cannot be reversed<sup>25</sup>.

### ***Antithrombin III Concentrate (AT III)***

Antithrombin III is used preoperatively to treat Heparin resistance or for VTE in primary or acquired AT III deficiency (Thrombate<sup>®</sup>)<sup>28</sup>.

### **Platelet aggregation inhibitors**

Platelet aggregation inhibitors are drugs that blocks platelet aggregation, activation or their adherence to the vessel walls.

#### ***Acetyl salicylic acid (ASA)***

Acetyl salicylic acid inhibits the endothelium's prostaglandin synthesis by irreversible inhibition of cyclooxygenase. Reduced prostaglandin levels prevent platelets to aggregate. Aspirin<sup>®</sup> and Trombyl<sup>®</sup> are two examples<sup>29</sup> of ASA.

#### ***Clopidogrel***

Clopidogrel causes an irreversible inhibition of the ADP receptor P2Y<sub>12</sub> on platelet membranes and thereby inhibits aggregation of platelets<sup>30</sup>. Plavix<sup>®</sup> is a well-used drug containing clopidogrel.

#### ***Ticagrelor***

Ticagrelor is an antagonist of the P2Y<sub>12</sub> receptor<sup>30</sup> that inhibits the platelet aggregation (e.g. Brilique<sup>®</sup>).

#### ***Prostacyclin (PGI<sub>2</sub>)***

Prostacyclin (PGI<sub>2</sub>) inhibits platelet activation and platelet adherence to the vessel walls. PGI<sub>2</sub> also acts as a vasodilator, e.g. epoprostenol<sup>31</sup> (Flolan<sup>®</sup>).

## **Thrombolytics**<sup>32</sup>

Clears and breakdowns fibrin clots. Used as a thrombolytic agent for massive DVT, PE, and AMI or to degrade occlusions in intravenous and dialysis catheters.

### ***Recombinant tPA***

Recombinant tPA catalyses the cleavage of plasminogen to plasmin, together with fibrin. It has a high affinity for plasminogen bound fibrin and breaks down the fibrin. Alteplas (Actilyse<sup>®</sup>) is a well-documented drug containing tPA.

### ***Urokinase***

Urokinase plasminogen activator (uPA) catalyses the conversion of plasminogen to plasmin and is used as thrombolysis in PE and AMI, e.g. Abbokinase<sup>®</sup>.

### ***Streptokinase***

Streptokinase is an effective and inexpensive fibrinolytic used as treatment in AMI and PE. Streptokinase is also secreted by streptococci and it activates plasminogen, e.g. Streptase<sup>®</sup>.

## **Procoagulants**

Substitutes coagulation factors, inhibits fibrinolysis, stimulates platelets or are antidotes to anticoagulants.

### ***Antifibrinolytics***

The most well known antifibrinolytic is tranexamic acid and it prevents fibrinolysis by competitively inhibiting the activation of plasminogen to plasmin<sup>33</sup>, e.g. Cyklokapron<sup>®</sup>.

### ***Prothrombin complex concentrate (PCC)***

Prothrombin complex concentrate is a plasma product containing vitamin K dependent proteins; FII, VII, IX and X. PCC treats bleeding due to deficiency of Vitamin K coagulation factors or reverse the effect of Warfarin<sup>34</sup>, e.g. Confidex<sup>®</sup> and Beriplex<sup>®</sup>.

### ***Vitamin K***

Vitamin K is used as treatment for bleedings caused by low prothrombin complex (F II, VII, IX and X). It is also an antidote to *VKA*: s<sup>35</sup>. Vitamin K<sub>1</sub> is Fytomenadion, which is distributed as Konakion<sup>®</sup>.

### ***Desmopressin Acetate***

Desmopressin Acetate releases FVIII and vWF from the endothelium, which stimulates the platelets to aggregate and shortens the bleeding time<sup>36</sup> (e.g. Octostim<sup>®</sup>, DDAVP<sup>®</sup>).

### ***Recombinant FVIIa (rFVIIa)***

Recombinant FVIIa is a very important drug for the treatment of FVII deficiency and Hemophilia. rFVIIa has also been used in trauma with conflicting results.<sup>37, 38</sup>, e.g. NovoSeven<sup>®</sup>RT.

### ***Protamine***

Protamine is an effective antidote to UNFH and LMWH. It works by electrostatic binding to these molecules and block their anticoagulant effect. Protamine is commonly used as reversal of UNFH at the end of heart surgery<sup>39</sup>, e.g. Protaminsulfat<sup>®</sup> Leo Pharma.

### ***Fibrinogen***

Fibrinogen is used for congenital fibrinogen deficiency and for fibrinogen consumption during for example ECMO<sup>40</sup>, e.g. RiaSTAP<sup>®</sup>.

### ***Recombinant FXIII (rFXIII)***

Recombinant FXIII is used for treatment of congenital factor XIII deficiency<sup>41</sup>, e.g. Fibrogammin P<sup>®</sup>.



## **Measuring coagulation and anticoagulation**

### **Activated Partial Thromboplastin Time (aPTT)<sup>42</sup>**

The Activated Partial Thromboplastin Time (aPTT) measures the activity of the intrinsic and common pathways of coagulation and is used for preoperative screening and monitoring of anticoagulative therapy. aPTT is sensitive to plasma levels of the contact factors XII, XI and FV, VIII, IX, X, prothrombin and fibrinogen. The term "partial" indicates that phospholipid is included but not Tissue Factor, which is necessary for activation of the extrinsic system. The term "thromboplastin" means the complex of the clotting factors that convert pro-thrombin to thrombin and the final synthesis of fibrin. Citrated platelet poor plasma (PPP) is warmed to 37°C. Phospholipid and a contact activator, e.g. kaolin, is added followed by calcium. The addition of calcium initiates the clotting and aPTT is the time from the addition of calcium to the formation of fibrin.

Reference value: 30-50 s

### **Activated Clotting Time (ACT)<sup>43</sup>**

Activated Clotting Time was first described by Hattersley in 1966<sup>44</sup>. It is a bedside or point of care test (POCT) of coagulation used to monitor the anticoagulant effect of UNFH in patients on bypass surgery, on ECMO, hemofiltration or hemodialysis. Fresh, whole blood is added to a tube containing a surface activator, such as kaolin or a glass. This results in the activation of coagulation by the contact pathway.

Reference value: 107 ± 13 s

Therapeutic range in CPB and heart surgery: 400-600 s

Therapeutic range in ECMO: 220-260 s

### **Anti-FXa<sup>45</sup>**

Anti-FXa is a test that measures the activity of heparin. Plasma is added to a reagent with factor Xa. Heparin from the patient binds AT III and inhibits FXa. The amount of Heparin in the patient's plasma is inversely proportional to the amount of remaining FXa. This can be used to calculate the anti-FXa level. Anti-FXa is useful in patients with heparin resistance or with an underlying condition that interferes with the aPTT test.

Therapeutic ranges:            LMWH: 0.5-1.2 IU/mL    UNFH: 0.3-0.7 IU/mL

Profylactic ranges:            LMWH: 0.2-0.5 IU/mL    UNFH: 0.1-0.4 IU/mL

### **Antithrombin III (AT III)**<sup>46</sup>

Antithrombin III is a test performed to test if patients are responding as expected to Heparin or if they are AT III deficient. In some patients with AT III deficiency, AT III needs to be given to reach a normal anticoagulant effect of heparin.

Reference value: Newborn: 60-90% Children and Adults: 80-120%

Plasma concentration: 0.15-0.2 mg/mL

### **Bleeding Time**<sup>47</sup>

The skin bleeding time is a measure of global hemostatic competence *in vivo*. A standardized incision is made in the subjects forearm (Surgicut<sup>®</sup> or Simplate<sup>®</sup> II). The specificity and sensitivity for Skin Bleeding Time (SBT) in von Willebrand Disease (vWD) is low as well as a predictor of surgical bleedings. It is seldom used clinically nowadays.

Reference value: 2-10 min.

### **Platelet Count (TPK)**<sup>48</sup>

The Platelet Count, or in Sweden Trombocyt Partikel Koncentration (TPK), is an assay that detect the amount of platelets in the blood. Acceptable level for normal hemostasis is considered to be  $50 \times 10^9/L$ .

Reference value:  $150-450 \times 10^9/L$

### **Prothrombine Time (PT)**<sup>49</sup>.

The Prothrombine Time (PT) measures the activity of the tissue factor pathway of coagulation and is used as preoperative screening, measuring of warfarin effect, liver dysfunction and vitamin K status. PT is sensitive to plasma levels of FVII, X, V, prothrombin and fibrinogen. Citrated PPP is incubated at 37°,  $Ca^{2+}$  in a phospholipid suspension is added to normalize the blood clotting ability and finally TF is added. The time is controlled from the addition of TF to the formation of fibrin.

Reference value: 12-13 s

### **International Normalized Ratio (INR)**<sup>50</sup> $(PT_{test}/PT_{normal})^{ISI}$

According to which test of PT is being used, different batches of manufacturers TF can vary. To overcome these differences the manufacturers assigns an International Sensitivity Index (ISI) value (ranging from 1-2). The INR is the ratio of a patient's prothrombine time to a control sample (normal) raised to the power of the ISI value for the used analytical system.

Reference value: 0.8-1.2 Therapeutic Interval: 2.0-3.5

## **Thromboelastography and Rotational Thromboelastometry (ROTEM®)\*<sup>51</sup>**

Thromboelastography (TEG) was developed by Prof. H. Hartert in Heidelberg during WWII<sup>52</sup>. In the 1980's the interest in TEG increased in USA for management of acute bleeding patients and in 1993 TEG became a trademark for an American Company. The ROTEM® system is an enhancement of TEG and was developed 1995-1997 in Germany. It includes an electronic pipette, four measurements channels and an integrated computer for automatic analysis. The citrated blood sample is in a cuvette where a cylindrical pin rotates. When the blood clots it limits the rotation of the pin more and more with rising clot firmness. This is detected optically and the computer calculates the ROTEM® curve and its numerical parameters. The curve is plotted two-sided and expressed in mm and time is calculated in seconds.

### ***Definition of ROTEM measurements***

#### ***EXTEM***

EXTEM examines the Extrinsic -Tissue Factor induced coagulation.

#### ***INTEM***

INTEM examines the Intrinsic- Contact induced coagulation.

#### ***FIBTEM***

In FIBTEM cytochalasin D blocks the platelets and only Fibrins contribution is analyzed.

#### ***CT (Clotting Time)***

Clotting Time is the time from start until initiation of clotting. The thrombin formation time (s).

#### ***CFT (Clot Formation Time)***

Clot formation time is the time when a clot size of 20 mm is detected. It measures both fibrin synthesis and reinforcement with platelets and FXIII (s).

#### ***MCF (Maximum Clot Firmness)***

The maximum clot firmness measures the stabilization by fibrin, platelets and FXIII (mm).

#### ***ML (Maximum Lysis)***

The maximum lysis is a measure of the reduction of firmness after MCF. ML <15% at 1 hour indicates a stable clot and ML >15% at 1 h indicates pathologic fibrinolysis.

\*(ROTEM® is a registered trademark of Tem innovations GmbH, Munich, Germany)

## Flow Chamber System<sup>53-55</sup>

A flow chamber is a device used to investigate *in vitro* thrombus formation under flow. By adjusting the flow rate (ml/h), the shear rate ( $s^{-1}$ ) can be varied to simulate venous or arterial flow conditions. On the flow chamber a coated cover slip is placed containing thrombogenic material. The most common coating material is collagen but also vWF, fibrinogen, fibronectin as well as endothelial cells and atherosclerotic plaque material can be used. To avoid clotting the blood is collected into  $Ca^{2+}/Mg^{2+}$  chelating anticoagulants such as citrate or ethylenediaminetetraacetic acid (EDTA). As the function of many plasma coagulation factors is  $Ca^{2+}$ -dependent the blood needs to be recalcified before entering the chamber to provide free ions for induction of thrombus formation. The thrombus formation is followed in real-time using a digital camera coupled to a microscope.

It is possible to measure platelet adhesion, aggregation and coagulation but also to determine the roles of platelet receptors and signaling proteins. Flow chambers are also excellent tools to analyze the intrinsic pathway of coagulation<sup>56</sup>.

The flow chamber used in this thesis consists of a transparent polycarbonate block with an engraved rectangular area (width 5 mm, height 50  $\mu m$ ). Inlet and outlet tubes are drilled at an angle of 20° to avoid flow disturbances.

## Hypovolemic Shock in Trauma<sup>57</sup>

### **Definition**

Shock is defined as an abnormality for the circulatory system that results in inadequate organ perfusion and tissue oxygenation. Shock can have different causes, hypovolemia, heart failure, CNS-injury or sepsis. In trauma, the by far most common cause of shock is hypovolemia and is in focus in this presentation. Causes of hypovolemic shock in trauma are injuries to blood vessels (arteries or large veins), highly circulated organs (the heart, lungs, liver, spleen or kidneys) and fractures of femur and pelvis. A ruptured ectopic pregnancy and burns can also result in hypovolemic shock. Cardiac Output (CO) is the volume of blood pumped by the heart per minute (L/min). CO is determined by multiplying the HR (beats per min) and the stroke volume (ml/beat). The stroke volume (amount of blood pumped with each cardiac contraction) is determined by, preload, myocardial contractility and afterload. *Preload* is mainly a result of the blood volume on the venous side. Normally approximately 70% of the body's blood volume is on the venous side and a major loss of the blood volume decreases preload and may cause a hypovolemic shock. *Myocardial contraction* drives the circulatory system and *Afterload* is the resistance to the forward flow of blood out of the pulmonary arteries or the aorta.

### **Pathophysiology**

Blood loss results in vasoconstriction and a decrease in visceral, cutaneous and muscle circulation. This response preserve blood supply to more vital organs like the kidneys, heart and brain. Endogenous catecholamine increases the peripheral resistance, which increases the diastolic blood pressure (DBP) and decrease the pulse pressure (PP). Breathing is affected with increased respiratory rate (RR) and with diminishing blood volumes urinary output (UO) decreases and the mental status is affected. Histamine, Bradykinin,  $\beta$ -endorphins and prostanoids (prostaglandins, prostacyklins and Thromboxanes (TXA<sub>2</sub>)) are other vasoactive hormones that are released into the circulation during shock. The earliest signs of bleeding are tachycardia and peripheral vasoconstriction resulting in, cool, whitened skin and extended transcapillary refill (TCR) > 2s. Low oxygenation of the peripheral organs induces anaerobic cell metabolism, which results in formation of lactic acid and metabolic acidosis. Prolonged shock with inadequate delivery of ATP destroys the cell membrane integrity and normal electrical gradient. Swelling of the endoplasmic reticulum, mitochondrial damage, lysosome rupture with release of cytotoxic substances soon follows. Sodium and water enters the cell and swelling starts. During hypovolemic shock Ca<sup>2+</sup> gets deposited inside cells.

Hemorrhage means an acute loss of circulating blood volume. The blood volume of an adult is approximately 7% of the body weight (e.g. 70 kg  $\approx$  5L) and for a child 80 ml/kg. Hemorrhage is divided in four different classes. The following estimated blood losses are for a 70 kg male.

## **Class I-IV Hemorrhage**

### ***Class I Hemorrhage***

In Class I Hemorrhage, up to 15% (750 mL) of the blood volume is lost. This results in minimal tachycardia, no change in blood pressure, pulse pressure, respiratory rate or urinary output. The patient gets slightly anxious.

### ***Class II Hemorrhage***

In Class II Hemorrhage, 15-30% (750-1500 mL) of the blood volume is lost. Tachycardia (HR 100-120) and tachypnea (RR 20-30) is present. The systolic blood pressure is normal but diastolic pressure is increased and the pulse pressure gets decreased. Urinary output is reduced to 20-30 ml/h. The patient is anxious, hostile or frightened.

### ***Class III Hemorrhage***

In Class III Hemorrhage, 30-40% (1500-2000 mL) of the blood volume is lost. Marked tachycardia and tachypnea (HR 120-140, RR 30-40) is present. There is a clear drop in systolic pressure and decreased pulse pressure. The urinary output is 5-15 ml/h and the patient can be confused. Class III hemorrhage requires blood transfusion and if necessary emergency surgery.

### ***Class IV Hemorrhage***

In Class IV Hemorrhage, > 40% (> 2000 mL) of the blood volume is lost. This is an immediately life-threatening situation. Marked tachycardia (HR >140) to bradycardia is noted. There is a significant decrease in the systolic blood pressure and a very narrow pulse pressure. The diastolic blood pressure may be impossible to measure. The skin is cold and pale and the urinary output is negligible. The patient is confused or lethargic. Class IV Hemorrhage requires rapid blood transfusion and immediate surgical intervention.

It must be remembered that the classification above is somewhat arbitrary. Trauma patients in bleeding shock can have both normal cardiovascular and respiratory status. There is no single factor (metabolic or physiological) that identifies all patients in hemorrhagic shock.

## **Treatment of hemorrhagic shock**

The treatment is directed to reverse the shock state by providing adequate oxygenation and appropriate fluid/blood resuscitation. Control of hemorrhage and restoration of adequate circulating volume is essential. Vasopressors are contraindicated because they worsen the tissue perfusion. See Damage Control Resuscitation and Damage Control Surgery below.

## **Trauma Induced Coagulopathy**

### **Definitions**

It is well known that severe trauma with hemorrhage induces coagulopathy. This is a state when the blood's ability to coagulate (form clots) is impaired. Massive blood transfusion (MBT) is required in 15% of multi-trauma patients but is inevitably followed by hypothermia and acidosis. This exacerbates the coagulopathy. The risk of dying is highly increased in coagulopathic patients<sup>58</sup> and patients receiving MBT<sup>59</sup>. Hypothermia (body temperature < 33 °C) and metabolic acidosis (pH<7.2) increases the mortality rate to 90%<sup>60-62</sup>. Trauma Induced Coagulopathy (TIC) is a complex process. It is only in the last years that the pathophysiology of TIC has started to be known and it seems to be the result of an imbalance of platelets, pro-coagulation, anti-coagulation, endothelium and fibrinolysis<sup>63</sup>. Acute Traumatic Coagulopathy (ATC) is a definition of the endogenous part of TIC. In clinical and laboratory studies, tissue injury alone does not lead to coagulopathy<sup>64</sup>. Tissue injury needs to be combined with hypoperfusion of organs to induce ATC<sup>65</sup>. ATC is probably a combination of factor V inhibition, low fibrinogen, anticoagulation, impaired platelet function and hyperfibrinolysis. ATC is aggravated by acidosis, hypothermia and hypo-coagulable fluids. This causes established TIC<sup>66</sup>. In the following text known factors of ATC will be discussed.

1) Definition of TIC: INR>1.2, aPTT> 40s, Platelets<120, ROTEM amplitude<35 mm at 5 min<sup>64,67</sup>.

### **Procoagulant impairment**

#### ***Factor V***

ATC develops rapidly after trauma with bleeding shock. A study of 45 trauma patients where coagulation was controlled at the scene, before fluid infusion and on hospital admission showed that 56% had abnormal coagulation 25 min after trauma<sup>68</sup>. In another recent study coagulation factors from 110 adult trauma patients with ISS > 15 were controlled. FII, V, VII, VIII, IX, X, XI and XII were analyzed. 22 of the patients had a critical coagulation factor deficiency (< 30%) but there were no total consumption of coagulation factors. All of the 22 cases had a deficiency of Factor V. Five patients had a critical deficiency in other factors<sup>69</sup>. FV is a central coenzyme that together with Ca<sup>2+</sup> helps FXa to convert pro-thrombin to thrombin. Activated Protein C (APC) causes FV inhibition. A conclusion of this study was that APC might have caused an isolated deficit of factor V.

#### ***Fibrinogen***

Fibrinogen declines rapidly after trauma. A prospective randomized study with 80 trauma victims compared coagulopathic and non-coagulopathic patients. It showed a significantly lower fibrinogen levels in patients with ATC (1.53 vs. 2.54 g/L,  $p<0.001$ )<sup>70</sup>. Fibrinogen is the central substrate for fibrin strands that reinforce platelet-clots and low fibrinogen level is an a predictor of mortality at both 24 h and 28 days ( $p<0.001$ )<sup>71</sup>.

## **Systemic anticoagulation and hyperfibrinolysis**

### ***Protein C***

ATC may be mediated by Protein C. A prospective observational study of 203 major trauma patients showed that combined severe traumatic injury and hypoperfusion activated the protein C pathway with coagulopathy as result. APC was significantly increased, factors V and VIII inactivated and fibrinolysis increased. Elevated APC-levels were associated with increased blood transfusion requirements, organ injury and mortality<sup>72</sup>. Another component of ATC is hyperfibrinolysis. The mortality rate in trauma patients with fulminant hyperfibrinolysis exceeds 80%<sup>73</sup>. Tranexamic acid is an antifibrinolytic drug that competitively inhibits the activation of plasminogen to plasmin. In a RCT in trauma patients the effects of tranexamic acid on death, blood transfusion and thrombotic events were analyzed<sup>74</sup>. More than 20000 patients in 274 hospitals were included and tranexamic acid made a safe reduction on the risk of death and is now recommended as a standard treatment for bleeding trauma patients.

### **Platelet dysfunction**

Platelet (PLT) count is reduced in trauma and the odds of death at 24 hours in massively transfused trauma patients was shown to decrease 12% for every  $50 \times 10^9/L$  increase in platelet count<sup>75</sup>. A high ratio of PLT to Packed Red Blood Cells (PRBC: s) improves outcome<sup>76</sup>. Other studies show conflicting results and it is not clear what is most important, the number or platelets or the *impaired platelet function* of the platelets. Further studies are needed.

### **Endothelial activation**

The Endothelium contributes to ATC. Thrombomodulin and protein C receptors are anchored to the capillary beds and capture thrombin thus accelerating protein C activity. APC inactivates FVa and FVIIIa but also consumes PAI, the major inhibitor of tPA. Traumatic hemorrhage and hypoperfusion leads to a vast release of tPA from the endothelium and this results in hyperfibrinolysis<sup>65</sup>. Another known anticoagulant event during trauma, apart from activation of PC, is auto heparinization, which is linked to endothelial glycocalyx degradation<sup>77</sup>. This degradation triggers for thrombin formation, protein C activation and hyperfibrinolysis.



## **Acidosis, Hypothermia, Hypocalcemia and Hemodilution**

Hemorrhage, exposure to low temperature, hypoperfusion and hypocoagulable fluids causes acidosis and hypothermia. For coagulopathy to develop the pH needs to be below 7.2 and the body temperature less than 33°C<sup>78</sup>. Hypothermia, 33-37°C, impairs platelet function and the formation of a platelet plug. Below 33°C, the enzyme activity is also strongly reduced, which contributes to coagulopathy<sup>79</sup>. Calcium is a necessary cofactor for the activation of several coagulation factors. Hypocalcemia is mainly the result of transfusion with citrated blood products, especially fresh frozen plasma (FFP). The faster the transfusion the faster the reduction in Calcium. S-Ca<sup>2+</sup> < 0.9 mmol/L shall be treated<sup>80</sup>. High volumes of crystalloid or colloid fluid administered to trauma victims are associated with worsened coagulopathy, increased requisite of blood products and an increased risk of MOF. To minimize the risk of iatrogenic induced coagulopathy, damage control resuscitation (DCR) is recommended<sup>64, 81</sup>.



## **Contemporary Trauma Surgery**

### **Advanced Trauma Life Support<sup>13</sup>**

Advanced Trauma Life Support (ATLS) is a concept of triaging trauma victims that was initiated after a tragic plane crash involving an orthopedic surgeon and his family in Nebraska 1976. The surgeon meant that he could provide better care to himself and three of his seriously injured children than the care they received at the primary care facility. He saw a need for a new trauma system and ATLS was developed. The concept has been spread around the world and today more than 50 countries use it to train doctors involved in trauma care. In ATLS injuries are prioritized and handled according to ABCDE. A means Airway with C-Spine protection, B means Breathing, C means Circulation, D is Disability and E stands for Exposure of the entire body. ATLS-courses provide lifesaving knowledge for the initial treatment of injured patients but do not teach definitive care.

### **Damage Control Surgery<sup>14</sup>**

Historically Navies used the term Damage Control as the emergency control of situations that might have caused the sinking of a ship. The term Damage Control Surgery (DCS) was popularized in trauma by Michel Rotondo in the 1990s<sup>82</sup> and refers to the rapid termination of an operation, after control of life-threatening bleedings and contamination, in favor of correcting the physiology in the ICU. Definitive treatment is delayed until the patient is stable and may be postponed for hours-days. One important goal is to correct the lethal triad of hypothermia, acidosis and coagulopathy. Examples of acute lifesaving DCS procedures are acute amputation of bleeding extremities, external stabilization of pelvic fractures, Pringles maneuver<sup>83</sup> or perihepatic packing of liver-injuries<sup>84</sup>, drainage of bile leakage, stapling and resection of injured bowel, acute splenectomy, shunting of the blood stream in injured blood-vessels, but also angiographic guided embolization of bleeding vessels. DCS has become the standard of treatment for severely injured patients worldwide<sup>85</sup> and in the year of 2000 more than 1000 patients had been treated with this concept<sup>86</sup>.

## **Damage Control Resuscitation**

In earlier ATLS manuals high volumes of crystalloids were recommended but during recent years it has become obvious that a restriction of i.v. fluids improve the care of lethally injured patients. This led to the concept Damage Control Resuscitation (DCR) and permissive hypotension. Since DCR was introduced there has been a fall in trauma related mortality<sup>87</sup>. The concept of permissive hypotension is transfusion of blood products to increase the blood pressure, not aiming for normotension but for a more cerebral alert patient. The goal is to keep SBP in penetrating trauma at 70-80 mm Hg, and SBP in blunt trauma at 90 mm Hg. The time of hypotension needs to be kept at a minimized with immediate transfer to the operating room.

### ***Damage Control Resuscitation***<sup>88</sup>

1. Permissive hypotension and hypovolemia. The systolic blood pressure should be kept at 80 mm Hg (90 mm Hg in associated brain injury).
2. Hemostatic transfusion (resuscitation) with FFP, PRBC, Platelets and Tranexamic Acid  
Avoidance of crystalloids and vasopressors.
3. DCS or Angiography to treat the hemorrhage.
4. Restoration of organ perfusion and oxygen delivery.

DCR is indicated in patients with hemorrhage class III and IV and a transfusion speed of more than 4 units of PRBC: s in the initial 2-4 hours.

## **Definitive Surgical Trauma Care**<sup>15</sup>

In 1993 five surgeons and members of the International Society of Surgery (ISS) and the International Association for Trauma Surgery and Intensive Care (IATSIC) met in San Fransisco at the meeting for American College of Surgeons (ACS). They had realized that there was a specific need for surgical training in trauma. It was believed that a short course focusing on life-saving techniques and surgical decision-making was required for surgeons, who dealt with major trauma on an infrequent basis. The course would meet a worldwide need that supplemented the ATLS-course. A curriculum and manual that forms the basis of the Definitive Surgical Trauma Care™ (DSTC) was approved in 1999 and the first manual was published in 2003. Since then the course has been delivered in more than 24 countries. In the DSTC course the following topics are covered. Physiology and the metabolic response to trauma, transfusion in trauma, DCS and the abdominal compartment syndrome, surgical decision making in major trauma and organ-orientated surgical techniques in trauma<sup>89</sup>.

## **Extracorporeal Membrane Oxygenation<sup>90</sup>**

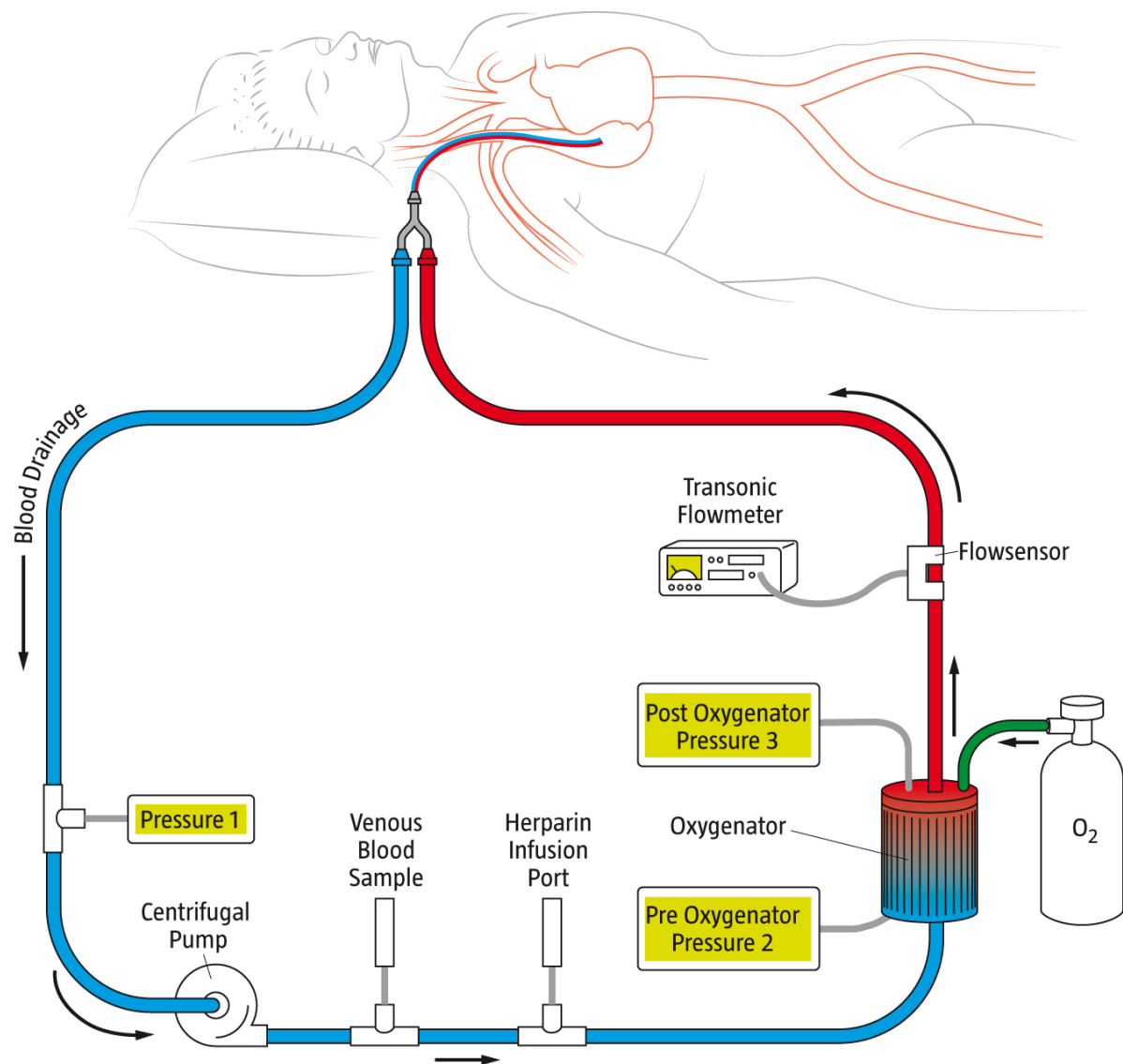
### **History of ECMO**

Extracorporeal Membrane Oxygenation (ECMO) literally means the oxygenation of blood on a membrane outside the body. ECMO can also be regarded as a modified heart-lung machine. The first known blood transfusions, between humans and lamb, were done by Richard Lower in 1629. Three hundred years later in 1953 the first heart surgery on cardiopulmonary bypass (CPB), an atrial septal defect, was performed by Dr. John Gibbon. In 1960 the first membrane oxygenators were introduced and during the years to come oxygenators and other devices were continuously improved. Silicone membrane oxygenators reduced the risk of hemolysis and the use of them led to the term ECMO. The first ECMO was performed in 1971 when Dr. JD Hill cannulated a motorbike victim with post-traumatic ARDS<sup>91</sup>. During this period Dr. Robert Bartlett, the father of modern extracorporeal support, was collaborating with engineering researchers. Bartlett was the first to use ECMO in children and neonates<sup>92</sup>. In 1975 a baby girl was born with meconium aspiration syndrome (MAS). Her mother was a refugee from Mexico and had abandoned her baby at the Californian border. Conventional intensive care treatment failed. Dr. Bartlett and his team treated her with ECMO for 72 hours. The little girl survived and the staff named her Esperanza, which is Spanish for “hope”. ECMO was brought to Stockholm in 1987 by colleagues Drs. Frenckner and Palmér and the first patient was a baby boy with Congenital Diaphragmatic Hernia (CDH). He survived and in September 2015 ECMO Center Karolinska had treated more than 1000 patients. ECMO is spreading around the world and centers are being established continuously. To date more than 69000 patients have been treated worldwide and there are more than 200 centers reporting to the ELSO registry<sup>90</sup>.

## ECMO physiology

Systemic Oxygen delivery ( $DO_2$ ) is the amount of oxygen delivered throughout the body each minute and the product of Cardiac Output (CO) and the arterial oxygenation. At rest  $DO_2$  is 5 times greater than the consumption ( $VO_2$ ) and in healthy subjects there is a cardiorespiratory homeostasis. When  $VO_2$  changes,  $DO_2$  changes as well. If cardiac output is decreased the oxygen increases, which will result in a decreased venous saturation.  $DO_2$  is controlled by CO, Hemoglobin (Hb) amount, Hb saturation and dissolved oxygen. Oxygen is present in blood; both dissolved in plasma and bound to red blood cells by hemoglobin. Hemoglobin bound  $O_2$  is critical for the oxygen transport because the amount of free dissolved  $O_2$  negligible in plasma. Each gram Hb can bind 1.34 ml  $O_2$ . The oxygen consumption can be calculated as the arterial-venous oxygen content difference time cardiac output. Red cells pass one at a time in the lung capillaries leaving  $CO_2$  and binding  $O_2$  through passive diffusion and the amount of oxygen absorbed is exactly equal to what is consumed in the metabolism.  $CO_2$  is excreted in the alveoli of the lung and is normally maintained at  $PCO_2$  40 mm Hg. The Cardiac Output is the result of preload, the heart rate, cardiac contraction, and the resistance in the arteries. The blood volume, vascular tone and blood viscosity (hematocrit) all regulate these factors. Hypovolemia (hemorrhage) results in a catecholamine mediated increased HR, raised contraction and vasoconstriction. Heart failure can be treated with assistance of these reflexes by increasing blood volume (transfusion), vasoactive drugs (e.g. norepinephrine and dopamine) or mechanical support (ECMO). When  $DO_2$  is severely decreased ( $DO_2/VO_2 < 2:1$ ) the metabolic demands are greater than the delivered oxygen and anaerobic metabolism leads to acidosis. The venous saturation in the Pulmonary Artery (PA) reflects the  $DO_2/VO_2$  ratio and is an important parameter for monitoring the ICU patients. If the oxygen extraction is 30% the venous saturation is 70% for example. In sepsis the  $VO_2$  is higher than normal and forces the  $DO_2$  to rise, to maintain the 5:1 ratio. ECMO can be used either as a pure lung support, veno-venous (VV ECMO) or if it bypasses the heart as veno-arterial (VA ECMO), meaning a full support of the heart and lungs. In the latter setting the patient's central circulation is maintained even if the native heart arrests. The ECMO Circuit consists of cannulas for drainage and infusion of blood, surface heparinized tubings, a roller- or centrifugal pump, a heater and the membrane-oxygenator (Fig. 7) Both in VV and VA ECMO blood is drained from the venous side, either from the right atrium of the heart or the inferior vena cava or both. Carbon dioxide is removed and oxygen is added through the membrane artificial lung. In VV ECMO, blood reenters the vascular system on the venous side of the heart, whereas in VA it enters in a major artery. In VA ECMO the total systemic blood flow is the sum of extracorporeal flow and the blood flow from the heart which means that the  $O_2$ - and  $CO_2$  content depends on how much each factor contributes. In VV ECMO the perfused blood is returned on the venous side and mixed in the right atrium, increasing the  $O_2$ - and reducing the  $CO_2$  level. Some of the venous blood will re-enter the ECMO system (referred to as recirculation) but the major part passes through the heart and lungs. If the lungs have active parts the saturation will increase further and the systemic circulation depends totally on the native heart function. Generally, indication for VV ECMO is resistant hypoxemia despite optimized ventilator management. VA ECMO is indicated when the former indication is combined with a persisting heart failure despite conventional treatment and vasopressor support.

## ECMO illustration



**Figure 7. ECMO-circuit (veno-venous).** Draining dual lumen cannula in the right atrium, venous blood (blue) is drained to a centrifugal pump, oxygenation and warming of the blood performed in the membrane oxygenator, oxygenated blood (red) is reinfused in the right atrium. Pressures are measured before the pump (P1), before the oxygenator (P2) and before the patient (P3). The blood flow is controlled with a flowmeter.

## **ECMO in trauma**

In 1971 Dr. J Hill was the first physician who used prolonged extracorporeal treatment outside of the operating room. The patient was a 24-year-old motorcyclist who had sustained a ruptured aorta and a post-traumatic acute respiratory distress syndrome (ARDS). The patient received venoarterial support for 75-hours and survived<sup>91</sup>. 5 years later Mattox reported of 39 patients whose critical condition prevented them to be transported to the operating room. Ten of the patients had massive traumatic thoracic injuries. They were supported with a portable cardiopulmonary bypass and in eight of the patients the hemorrhage could surgically be controlled, and the bypass discontinued<sup>93</sup>. After this the literature concerning ECMO and trauma was sparse for almost twenty years. There are three major reasons imaginable for this. Firstly, the ECMO systems have been considered to be too complicated, time consuming and difficult to connect to the patient. Secondly, extracorporeal oxygenation has traditionally been associated with systemic heparin, which obviously is contraindicated in the traumatized patient. Thirdly, few centers have had a team on 24h-7d stand by for emergency cannulations. In 1995 Perchinsky et al reported on six massively injured patients who received ECMO without systemic heparin. Three of the patients survived and they concluded that ECMO with heparin-bonded circuits could offer safe support while the primary injuries were being evaluated and treated<sup>94</sup>. From the end of the 1990s and forward reports on ECMO after trauma has been more frequent. Michaels et al reported, in 1999, of 30 patients where ECMO was safely used in trauma patients with multiple injuries and severe pulmonary failure. Early implementation of ECMO was associated with improved survival<sup>95</sup>. The positive effects of ECMO in posttraumatic ARDS have been verified in additional reports and can no longer be regarded as controversial<sup>96-100</sup>. The use of ECMO in trauma with hemorrhagic shock is uncommon and somewhat controversial.

In 2010 Dr. Matthias Arlt and his group in Regensburg were the first to report on a series of ECMO treated adult trauma patients with bleeding shock<sup>101</sup>. Their results have initiated a new way of thinking. The application of the extracorporeal circuit, not only for gas exchange, but also as heart and lung support in a critical state with hemorrhagic shock and coagulopathy opens for new, unknown effects and indications. Dr. Arlt and his group treated ten severely injured trauma patients, with a mean ISS of over 70. Seven received VV ECMO and three VA ECMO. Cardiopulmonary failure was effectively stabilized. Systemic gas exchange and blood flow was rapidly improved within 2 h on ECMO. The bypass was combined with a specially designed high-speed fluid line, which enabled fast high-volume transfusion. Six patients survived and recovered completely. The increasing number of trauma patients treated with ECMO raises questions about correct indications but also effects and results of the treatment. There are very few animal studies performed in this field. When is ECMO indicated in trauma? What are the positive effects? Which negative effects and risks exist? What happens to the central circulation and how is coagulopathy affected? The main purpose of this thesis was to address some of these questions.



## **Blood activation on artificial surfaces and in extracorporeal circuits**

### **Introduction**

Within the blood vessel, the endothelial cells and the blood function together in a perfect balance, or hemostasis, but when blood is exposed to artificial biomaterials, like stents, grafts and cannulas, plasma proteins immediately get adsorbed to the foreign surface and a new interface is created between the surface and the blood. In the ECMO circuit, the circulation further challenges the blood by turbulence, shear stress, osmotic forces and cavitation and this increases the activation. Proteins in the complement system and contact pathway of coagulation will be bound or inserted into the protein film on the surface. The proteins generate mediators that activate leukocytes, monocytes and platelets. The anaphylatoxins C3a and C5a, the lytic sC5b-9 complex from the complement system, bradykinin and thrombin from the contact pathway are important examples. These mediators trigger leukocytes, monocytes and platelets leading to inflammation, capillary leakage and thrombotic reactions. The process is directed against the foreign material but the activated systems can lead to a strong inflammatory response that results in a capillary leakage that can cause dysfunction in all organs. The inflammatory response mimics both the systemic inflammatory response syndrome (SIRS) and the acute respiratory distress syndrome (ARDS). This may worsen the situation for an already compromised patient. Thromboembolic complications following the activated coagulation cascade can also injure the patient or even become fatal.

The following text focuses on how the coagulation system is activated on artificial surfaces such as ECMO. Artificial surfaces induces in several processes, protein adsorption, adhesion of platelets, thrombin synthesis and activation of the complement system.

### **Protein adsorption<sup>102, 103</sup>**

Blood is a mixture of cells and plasma. The major constituents of plasma are proteins and the major protein is albumin. The first event in thrombus formation on artificial surfaces is the adsorption of plasma proteins. Proteins can interact and undergo conformational changes in structure and that is an important factor for protein adsorption. This may also change the proteins biological activity. Fibrinogen and von Willebrand factor adsorb to the protein surface and facilitates platelet adhesion. Fibrinogen is replaced by FXII, HK, PPK and FXI, the contact pathway factors. Activation of FXII both triggers thrombin generation and activates the complement system. The complement and coagulation system cross talk and thrombin generation is amplified.

## **Cell adhesion** <sup>104, 105, 106</sup>

Adsorbed proteins induce adhesion of platelets, leukocytes and erythrocytes to the artificial surfaces. Fibrinogen is the central protein responsible for platelet adhesion. The adsorption of fibrinogen is greater on hydrophobic than hydrophilic surfaces. Artificial surfaces activate platelets and they release TXA<sub>2</sub> and ADP. TXA<sub>2</sub> and ADP increase the platelet response on the surface. This results in platelet thrombus formation and with prolonged exposure of artificial surfaces to blood the circulating platelet count decreases. During ECMO perfusion the amount of circulating platelets are reduced due to adhesion and aggregation. Platelets are also injured but the effects of activation outweigh the injuries. A high shear rate increases the platelet deposition and generates FXa from TF: FVIIa. Lower shear rates reduce platelet deposition but instead increase fibrin deposition. A low pump speed increases the risk of thrombosis and clotting in the extracorporeal circuit. Leukocytes also adhere to fibrin, platelets and activated complement components. The leukocytes generate superoxide and free radicals but also release PAF, Interleukins and Tumor Necrosis Factor (TNF). These substances further activate platelets and induce TF expression from Monocytes. Erythrocyte adhesion is passive and not depending on receptor-mediated adherence as thrombocytes and leukocytes. Erythrocytes develop reversible echinocytic changes but can also be hemolyzed by shear forces and activated complement<sup>107, 108</sup>. Roller pumps cause more hemolysis than centrifugal pumps<sup>109</sup>. Erythrocytes can release ADP, especially during high shear stress with hemolysis, which further activates platelets.

## **Contact activation**

The contact system factors adsorb to foreign surfaces and facilitate activation of the intrinsic pathway of coagulation. Several studies have indicated that catheters promote clotting via the contact system both *in vitro*<sup>110, 111</sup> and *in vivo*<sup>112</sup>. FXII is auto activated by negatively charged ions and FXIIa activates PPK and FXI. HK binds to the surfaces and acts as a cofactor, increasing the FXII activation. PPK activation results in Kallikrein generation, which activate more FXII. Activated FXI generates thrombin and results in fibrin and local platelet aggregation. Activated platelets further activate the coagulation system. Fibrin reinforces the platelet aggregates and can create a mixed platelet and fibrin thrombus. Such a thrombus can eventually cause the medical device to fail or separate and embolize to critical organs like the brain, lungs, heart or kidneys.

## Complement activation

The complement system is also activated when blood gets in contact with the extracorporeal circuit or the oxygenator<sup>113</sup>. The artificial surfaces generate PK which cleaves FXIIa to  $\beta$ -FXIIa.  $\beta$ -FXIIa activates the C1 in the classical pathway. C3 and C5 adhere to artificial surfaces and are also activated by PK. C3a and C5a attract leukocytes and promote surface activation. The fibrinolytic system is early activated during ECMO<sup>114</sup>. Circulating thrombin stimulates endothelial cells to produce tPA, which cleaves plasminogen to plasmin. Plasmin causes fibrin-dissolvement and inhibits fibrinogen and V, VII, IX and XI. The cleavage reaction produces D-dimers, which are used as markers of clotting and fibrinolysis during ECMO<sup>115</sup>.

## Summary

As a summary thrombus formation on artificial surfaces are the result of a combination of protein adsorption, cell-adhesion, platelet activation and aggregation. Contact initiated FXIIa induces thrombin generation and fibrin synthesis. The complement system is closely related to coagulation and is also activated on artificial surfaces. In ECMO the endogenous antithrombotic activity becomes overwhelmed and makes systemic anticoagulation necessary to withhold the extracorporeal circuits integrity (Fig. 8).

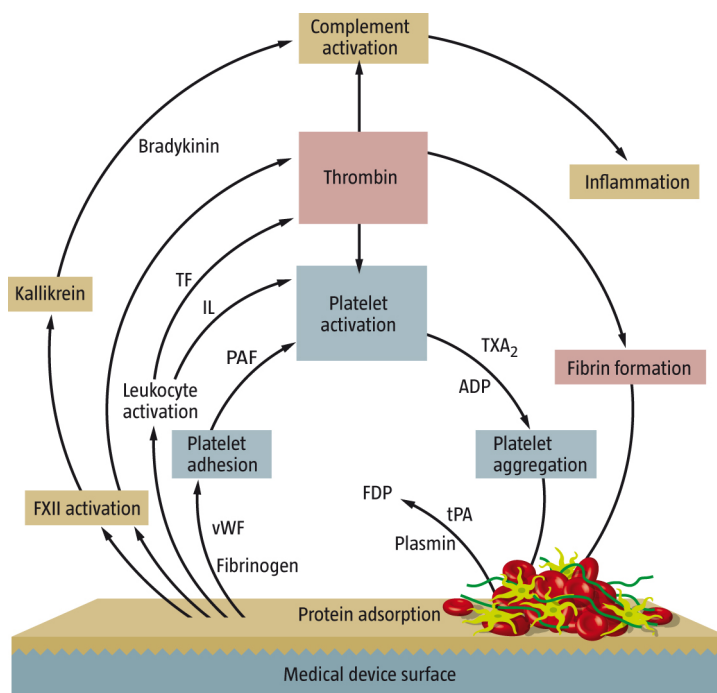


Figure 8. Blood activation in the extracorporeal circuit.



## **Anticoagulation and blood-contacting medical devices**

Failure of cannulas and other medical devices is commonly due to clotting. This is caused by a combination of poor surface biocompatibility and flow disturbance. Improved hemodynamics decreases flow disruption and the risk of platelet activation. There are basically two methods of preventing thrombosis/clotting in blood-contacting medical devices. Synthesis of biomaterials with less thrombogenicity and systemic administration of anti-coagulants or anti-platelet drugs.

### **Synthesis of less thrombogenic biomaterials**

#### ***Reduced protein/cell adsorption***

*Polyethylene oxide (PEO)*<sup>116</sup> contains hydrophilic ether oxygen and adsorbs less proteins and cells than other polymers. In vitro studies are positive but there are no studies in human so far. *Albumin coated surfaces*<sup>117</sup> adheres less platelets than fibrinogen and  $\gamma$ -globulin and has been covalently grafted onto artificial surfaces. Vascular grafts and mechanical heart valves have been coated with *pyrolytic carbon*<sup>118</sup> and this has reduced initial platelet adhesion, but long-term patency is similar to uncoated and patients still need lifelong anti-coagulation with vitamin K antagonists. *Phosphorylcholine surfaces*<sup>119</sup> resist protein and cell adhesion and have shown reduced platelet adhesion in animal models. Elastin is a fundamental part of the vessel wall and it reduces platelet adhesion. *Elastin inspired polymers*<sup>120</sup> have now been linked to silicone with decreased fibrinogen and immunoglobulin adsorption as a result. Passive adsorption of an elastin polypeptide also resulted in a reduction of platelets deposited *in vitro*. The elastin-inspired polymers are promising new biomaterials.

### **Inhibition of thrombin generation and fibrin formation**

#### ***Endothelial cells***

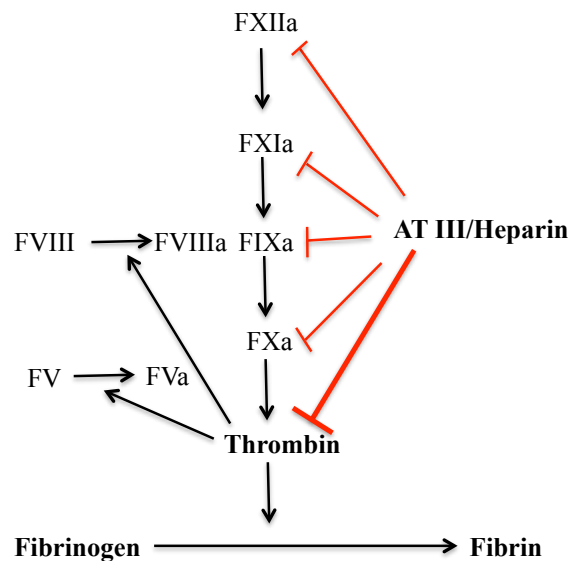
Endothelial cells have by nature anti-coagulant attributes that make them non-thrombogenic. Attempts to imitate endothelial cells have been made. Artificial surfaces have been seeded with endothelial cells with limited results. Biomaterials with bioactive molecules such as *Corn Trypsin Inhibitor (CTI)*<sup>121</sup>, heparin, and direct thrombin inhibitors have been more successful. A combination of *Polyethylene glycol (PEG)* and *CTI* coated surface both reduced fibrinogen adsorption and inhibited FXII autoactivation, reduced thrombin generation and prolonged the clotting time in plasma. *Heparin*<sup>122</sup> and *Heparin-AT* have been coated on biomaterial surfaces. Results from animal models are promising, but RCTs, angiographic and clinical endpoints have not shown any difference to uncoated surfaces<sup>123</sup>. Alginate-heparin composites on the surface of polyvinylchloride (PVC) may be more efficient in preventing thrombosis. *DTIs* (hirudin, bivalirudin and argatroban) have been grafted on surfaces to inhibit thrombin. They bind to thrombin directly in a 1:1 fashion. DTIs have been grafted onto albumin surfaces but their durability is questionable and *in vivo* data are limited. *Thrombomodulin*<sup>124</sup> is a protein normally expressed on the surface of endothelial cells. It is a cofactor to thrombin and converts thrombin from a pro-coagulant to an anti-coagulant enzyme. Thrombomodulin activates protein C, which in turn inactivates FVIIIa and FVa. Only a few animal studies have been done with recombinant thrombomodulin bound to polyvinyl and polyurethane surfaces. *Aspirin* and

*Clopidogrel* still are the main used anticoagulants for prevention of stent thrombosis and Acute Coronary Syndrome (ACS). They act through inhibition of platelet aggregation and to imitate their effect  $PGI_2$  and  $PGE_2$  have been connected to albumin-surfaces. Finally biomaterials that release *Nitric Oxide (NO)*<sup>125</sup>, a potent inhibitor of platelet aggregation, have been synthesized. One limitation with NO releasing materials has been the relatively thick coating material necessary.

### Systemic administration of antiplatelet drugs or anticoagulants

#### *Heparin*

Optimized hemodynamics and surface-bound anticoagulation is at present not enough to prevent thrombosis in extracorporeal circuits and oxygenator membranes. The by far most used and effective systemic anticoagulant, during CPB and ECMO, is unfractionated heparin (UNFH). UNFH activates AT III and increases its potency a thousand fold. AT III inhibits thrombin, FXa and other coagulation factors. (Fig.9). UNFH creates an increased risk of hemorrhage, which sometimes might be fatal. In ECMO it is mainly the contact pathway that is activated. Therefore less anticoagulation is required than in CPB and heart surgery, where the tissue factor pathway also is activated.



**Figure 9. Unfractionated Heparin** amplifies AT III and inhibits Thrombin and the intrinsic coagulation factors.

### ***Indirect FXa inhibitors, Low Molecular Weight Heparin and Direct Thrombin inhibitors***

Fondaparinux, is an indirect FXa inhibitor and Dabigatran is an oral thrombin inhibitor. They both have limited capacity to inhibit catheter-induced clotting and they are both less effective than UNFH and Warfarin<sup>126-128</sup>. Enoxaparin is a LMWH and has an effect on catheter-induced clotting, intermediate between fondaparinux and UNFH<sup>110</sup>. Long heparin chains can inhibit clotting in catheters better than short chains. This is because long chains can simultaneously bind to AT III, FXII, FXI, FIX and FX, forming more stable enzyme-inhibiting complexes. Fondaparinux only binds AT III and, without inhibiting the upstream enzymes; catheters can induce FXa generation that overwhelms Fondaparinux. Enoxaparin can bridge the up-stream enzymes and is superior to fondaparinux.

### **Novel strategies in anticoagulation of medical devices**

The aim of modern biomaterial research is to synthesize surfaces that reduce the risk of thrombus formation. Modern artificial surfaces are more resistant to protein- and cell adhesion. The protein adsorption and the activation of FXII are the primary causes of clotting in blood containing medical devices. In the current situation Heparin is needed either as surface bound- or systemically to prevent clotting in CPB and ECMO systems. An increased understanding of the contact pathway and complement activation in blood-contact medical device thrombosis may open new possibilities for both systemic and local approaches. Targeting the up-stream enzymes of contact activation, FXII and FXI, offers interesting anticoagulation strategies for the future.





## **2. AIMS OF THE THESIS**

### **Overall aims**

**The overall aims of this thesis were:**

- To achieve higher survival for trauma patients in hypovolemic shock.
- To develop safe anticoagulation, when treating trauma patients on ECMO.
- To develop a novel strategy for topical hemostatics.

### **Specific aims**

**The specific aims of this thesis were:**

- I) To study how central venous and arterial pressures alters during ECMO.
- II) To investigate if a monoclonal antibody directed against coagulation factor XIIIa could prevent clotting in an ECMO system with preserved hemostasis.
- III) To study how ECMO affects coagulopathy in class IV shock.
- IV) To investigate how Polyphosphate induces hemostasis in a grade II liver injury.



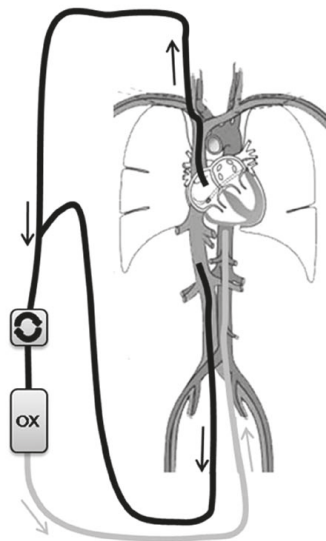
### 3. MATERIAL AND METHODS

The studies we have performed had not been possible without our study animals. The most sophisticated computers and software are unable to process the complicated experiments and interpret the full physiology of living animals. Therefore living species were unavoidable. The Ethics Committee for Experiments in Animals, Karolinska Institutet, Stockholm, Sweden approved all studies and all animals have been treated like individuals with respect and carefulness.

#### Study I

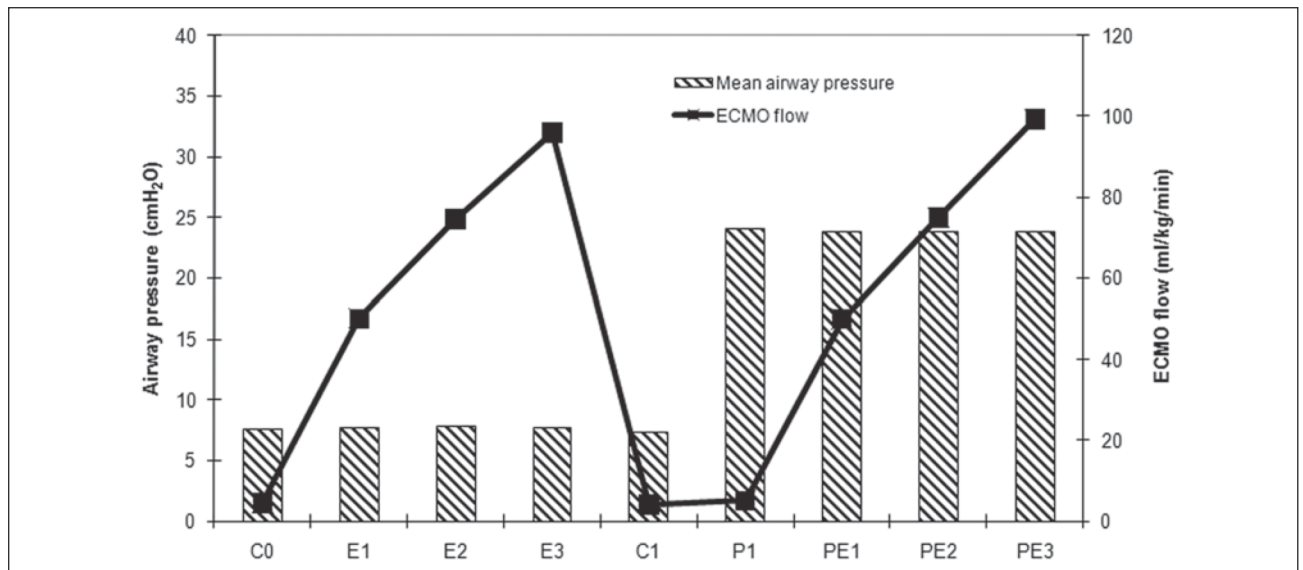
In study I we were interested in how VA ECMO would affect the central venous pressure and mean arterial pressure both in a hemodynamic normal state and a setting of increased central venous pressure and right ventricular load, mimicking acute lung injury.

Ten swine (50% Yorkshire and 50% Swedish Landrace) were anesthetized and cannulated for venoarterial ECMO with venous cannulas for drainage in the right atrium and the inferior vena cava (IVC), and arterial cannula for infusion in the abdominal aorta (AA). The animals were connected to an ECMO system with a roller pump, a heat exchanger and a membrane oxygenator (Fig.10). It was custom built and identical to the clinical used models, apart from replacement of surface-heparinized tubing with standard tubings for economic reasons. Four different flow rates were used during the experiments,  $5 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  (C0),  $50 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  (E1),  $75 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  (E2) and  $100 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  (E3). Central venous pressure in the superior vena cava ( $\text{CVP}_{\text{sup}}$ ) and the inferior vena cava ( $\text{CVP}_{\text{inf}}$ ), mean arterial pressure (MAP), portal vein pressure ( $\text{P}_{\text{port}}$ ) and portal vein flow ( $\text{Q}_{\text{port}}$ ) were monitored and recorded continuously using a computer-based data acquisition system.



<u>ECMO With normal circulation</u>	<u>Flow Rate</u>
<b>C0:</b> Control 0 without intervention	5 ml/kg/min
<b>E1:</b> ECMO 1 without intervention	50 ml/kg/min
<b>E2:</b> ECMO 2 without intervention	75 ml/kg/min
<b>E3:</b> ECMO 3 without intervention	100 ml/kg/min
<u>ECMO With increased right ventricular load</u>	<u>Flow Rate</u>
<b>C1:</b> Control 1 without intervention	5 ml/kg/min
<b>P1:</b> Increased ventricular load	5 ml/kg/min
<b>PE1:</b> ECMO1 + increased ventricular load	50 ml/kg/min
<b>PE2:</b> ECMO2 + increased ventricular load	75 ml/kg/min
<b>PE3:</b> ECMO3 + increased ventricular load	100 ml/kg/min

**Figure 10. Venoarterial ECMO.** Venous blood is drained from the right atrium and inferior vena cava. Oxygenated blood reinfused in the abdominal aorta. Roller pump and oxygenator indicated. Arrows are reflecting the blood flow. The table illustrates the different settings of ECMO flow and right ventricular load.



**Figure 11. ECMO blood flow and mean airway pressures reflecting the study protocol.** Hemodynamic measurements were made a steady state at each point seen on the horizontal axis. C0 and C1 are controls without any intervention. P1 is a measurement with increased right ventricular load (for details see Methods) without ECMO. E1-E3 and PE1-PE3 are measurements with increasing ECMO flow support in three steps with normal and increased right ventricular load, respectively. Courtesy of Michael Broomé (Fig. 10 and 11)

Hemodynamic steady state was reached within less than 5 minutes at each flow level. The flow levels were chosen as realistic levels of circulatory support in swine with expected cardiac outputs of 125-150 ml/kg/min, allowing some residual pulmonary circulation to avoid clot formation. Measurements were made not less than 10 minutes after change of flow. After minimizing ECMO flow, a new control (C1) was done to test the stability of the model. The next measurement (P1) was done after inducing a substantial increase in right ventricular load and venous pressures, induced by increasing positive end-expiratory pressure (PEEP) levels on the ventilator from 5 to 15-20 cm H<sub>2</sub>O and rapid infusion of 2-5 ml/kg of a dextran solution (Fig. 11). The endpoint for this intervention was a CVP<sub>sup</sub> of 15 cm H<sub>2</sub>O and a substantial decrease in MAP to about 60 mm Hg. After this measurement, the ECMO flow was increased stepwise to the same levels as in the first stage of the study while maintaining the ventilator settings and the volume load to simulate a clinical situation with increased right ventricular load. Measurements were at each level of ECMO flow (PE1-PE3) as before.

## **Study II**

In study II a recombinant fully human antibody (3F7) that binds into the FXIIa enzymatic pocket was investigated. Of particular interest was if 3F7 could provide thromboprotection in the extracorporeal circuit as efficient as Heparin, but without impairing the hemostatic capacity. An ECMO-system that is normally used for pulmonary and circulatory support for infants was adapted for rabbits. 15 New Zealand White rabbits were divided in three groups with 5 rabbits in each group. The control group received Saline instead of anticoagulation, the Heparin group received unfractionated heparin (UNFH) in identical doses as those used in ECMO-patients and the Test group received 3F7 (7mg/kg).

### **Thromboprotection**

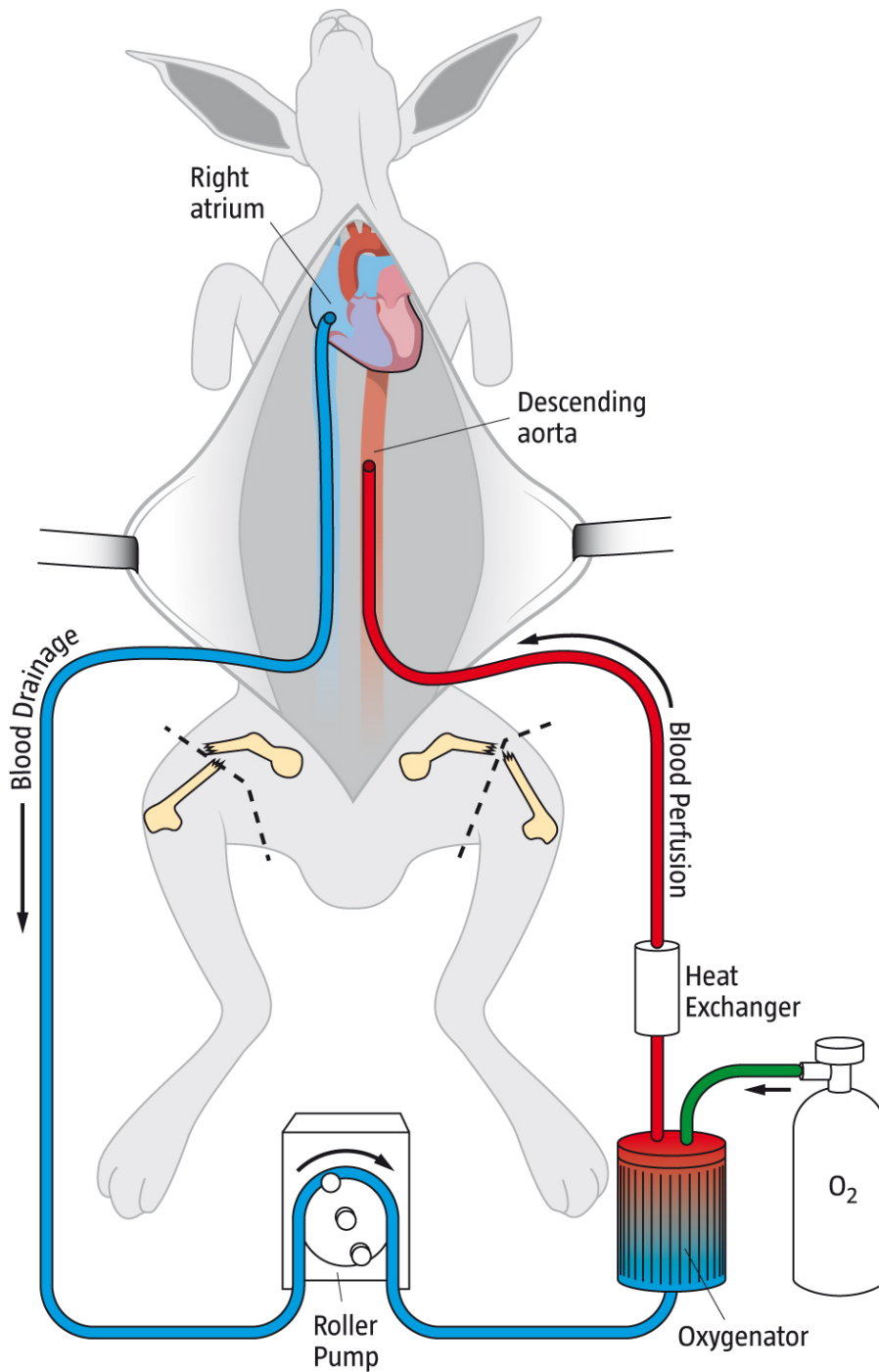
In the circuit, the oxygenator is the most critical part for clotting (thrombus formation). The blood pressure gradient between the inlet and outlet of the oxygenator was controlled as a measure of occlusive thrombus formation. The blood clotting in the oxygenator was also controlled with Scanning Electron Microscopy (SEM) after 6 hours of ECMO. The amount of fibrin deposited in the oxygenator was quantified both visually from high power field images (HPF) and by Western blotting using a fibrin-specific antibody.

### **Bleeding**

The rabbits hemostatic capacity were analyzed via incision-triggered bleeding times and blood loss from injury site at the end of the ECMO procedure. We used skin-bleeding time in the ear (Surgicut<sup>®</sup> jr), cuticle bleeding time and cuticle bleeding volume.

### **Study III**

In study III it was investigated how VA ECMO affects Traumatic Induced Coagulopathy (TIC). 15 New Zealand White Rabbits were used in an experimental trauma model with bilateral femur fractures, laparotomy and a class IV bleeding shock (>40% hemorrhage of blood volume). The hemorrhagic shock lasted for 90 minutes. Five of the study rabbits, the control group, were treated with a standard transfusion protocol (transfusion of whole blood, calcium and active rewarming). Another five rabbits, the ECMO group, received the same treatment as the former group but were also on venoarterial ECMO for 60 minutes (Fig.12). The ECMO system was designed as described in Study II. Finally five rabbits were used as blood donors for priming the ECMO-circuits (one rabbit/circuit). The circulatory status (HR, MAP), acid-base balance (pH, Lactate and BE) and temperature were controlled every 15 minutes. The coagulation capacity and bleeding were controlled at base line (0 min), after 90 minutes of bleeding shock and after another 60 min of resuscitation (at 150 min). Coagulation was controlled with rotational thrombelastometry (ROTEM), platelet count, aPTT, INR and fibrinogen. In vivo bleeding was controlled with ear bleeding time and cuticle bleeding time.



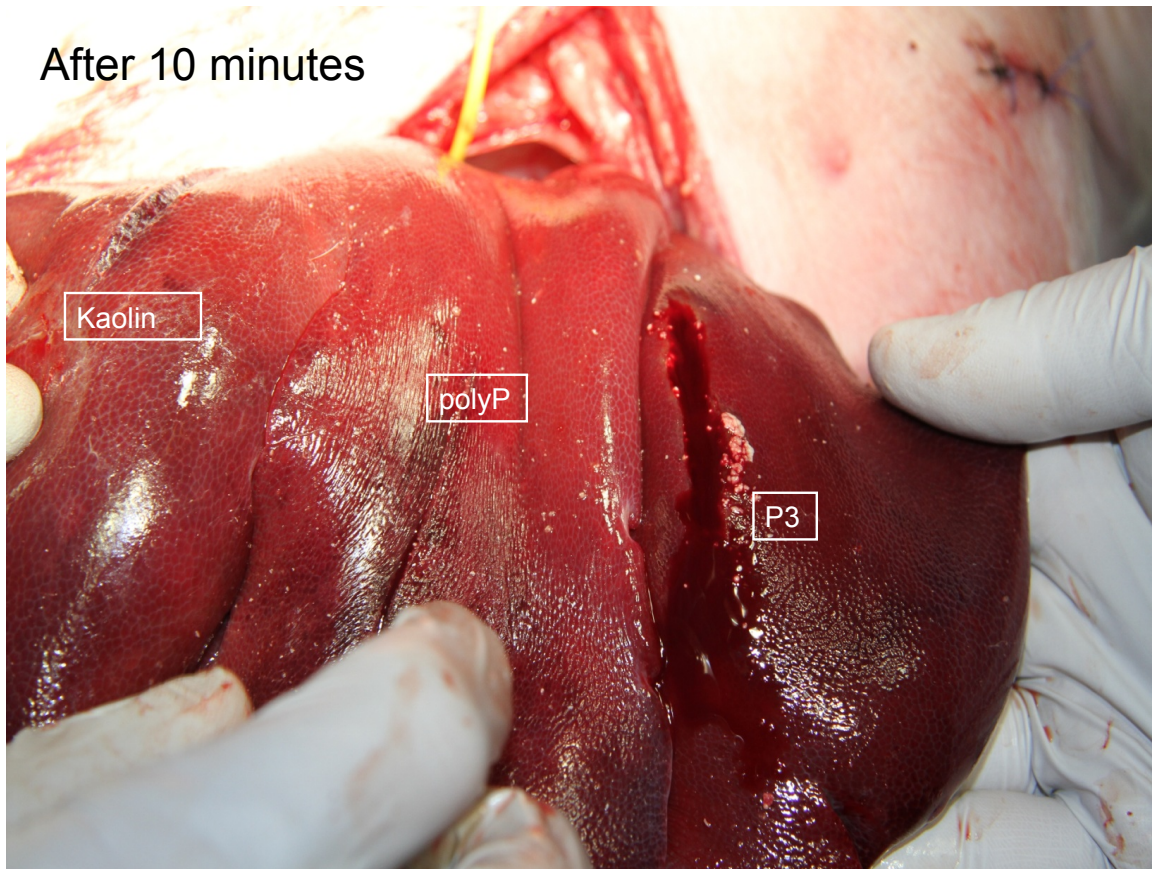
**Figure 12. The Rabbit ECMO-Trauma model.** The animals sustained laparotomy and bilateral femur fractures and were exsanguinated to class IV Shock. The hemorrhagic shock lasted for 90 min and the following resuscitation for 60 min. The ECMO circuit: Venous draining cannula in the right atrium (blue), roller pump, heparinized membrane oxygenator for saturation and carbon dioxide removal, water heat exchanger (38.5°C). Blood is reinfused in the descending aorta (red).

## Study IV

In study II the antibody 3F7, which *systemically inhibits FXIIa*, was evaluated according to its thromboprotective effect in an ECMO system. A severely injured patient on ECMO may still benefit of a *local activation of FXII* for clotting. Polyphosphate (PolyP) is a linear molecule consisting of  $\text{PO}_4^{2-}$  (phosphate-ion) chains and it is released from platelets when they are activated in vascular injuries. PolyP has hemostatic properties by initiating the intrinsic pathway of coagulation. PolyP activates FXII, the Hageman factor. In study IV PolyPs hemostatic effect in vivo on grade II liver injuries in swine were controlled. The synthetic PolyP we used resembled platelet PolyPs and had a chain length of 70-100 units. Ten swine were used in the study. Incisions in each of three liver lobes were performed (3 cm long and 1 cm deep). The amount of bleeding from each liver injury was controlled to be macroscopically equal and then Total Vascular Exclusion (TVE), by occlusion of the infra- and suprahepatic IVC, the portal vein and hepatic artery (Pringle's maneuver) was performed for 3 minutes. During this time the liver wounds were not bleeding at all and meanwhile three different agents in powder form were applied. 500 mg Kaolin (K), triphosphate (P3) or PolyP were used. The surgeon was blinded to the powder type. The wounds were randomly filled ensuring that one powder was not always placed in the same injured liver-lobe. After 3 minutes the liver-circulation was restored. All wounds were compressed manually for 10 minutes including the time for TVE (Fig.13). When the pressure was relieved the bleeding was photographed and the macroscopic coagulation was judged to be non-bleeding (0), minor bleeding (1) or major bleeding (2). Major bleeding was defined as when the rate of bleeding was the same as before the hemostatic agent was applied. The compress material from each wound was weighted. The hemoglobin from each wound was measured with spectrophotometric absorbance and the true blood-loss from each injury was calculated. Before termination of the swine thorough excisions of the liver-wounds were made to obtain specimens for immunohistochemistry and electron microscopy. The liver excisions were made at two time points, 30 min and 4 h after injury, and after this the animals were sacrificed.



After 10 minutes



**Figure 13. The liver injury model.** Grade II liver injuries were treated with Kaolin, PolyP and P3 respectively. Bleeding was visually registered after 10 min of compression.



## 4. RESULTS AND DISCUSSION

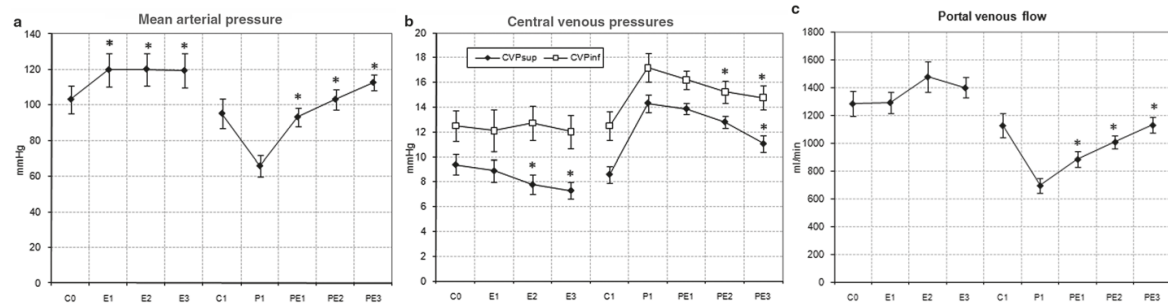
### Study I

#### Results study I Part I. Effects of ECMO in a normal hemodynamic state: C0-E3

Veno arterial ECMO increased MAP and reduced  $CVP_{sup}$ , while HR,  $Q_{port}$ ,  $P_{port}$  and  $CVP_{inf}$  did not change significantly. Central venous oxygen saturation ( $SvO_2$ ) increased and end tidal carbon dioxide ( $etCO_2$ ) decreased reflecting an increase in systemic perfusion with reduced pulmonary blood flow (Fig.14).

#### Results Study I Part II. Effects of ECMO with increased right ventricular load: P1- PE3.

Venoarterial ECMO increased MAP and  $Q_{port}$  and reduced both  $CVP_{sup}$  and  $CVP_{inf}$  while HR and  $P_{port}$  remained unchanged during the experimental stage with increased right ventricular load.  $SvO_2$  increased and  $etCO_2$  decreased as in part (Fig.14).



**Figure 14 abc. Hemodynamic data reflecting changes induced by increasing ECMO flow.** Mean arterial pressure increased significantly both during a normal circulatory state (Fig.14a, E1-E3) and with increased right ventricular load (Fig.14a, PE1- PE3). Similar circulatory changes are seen in portal venous flow (Fig.14 c, E1-E3 and PE1-PE3). Central Venous Pressure is decreased with increased ECMO flow during both stages (Fig. 14 b, E1-E3 and PE1-PE3), reflecting the unloading of the venous system. Courtesy of Michael Broomé.

## Discussion study I

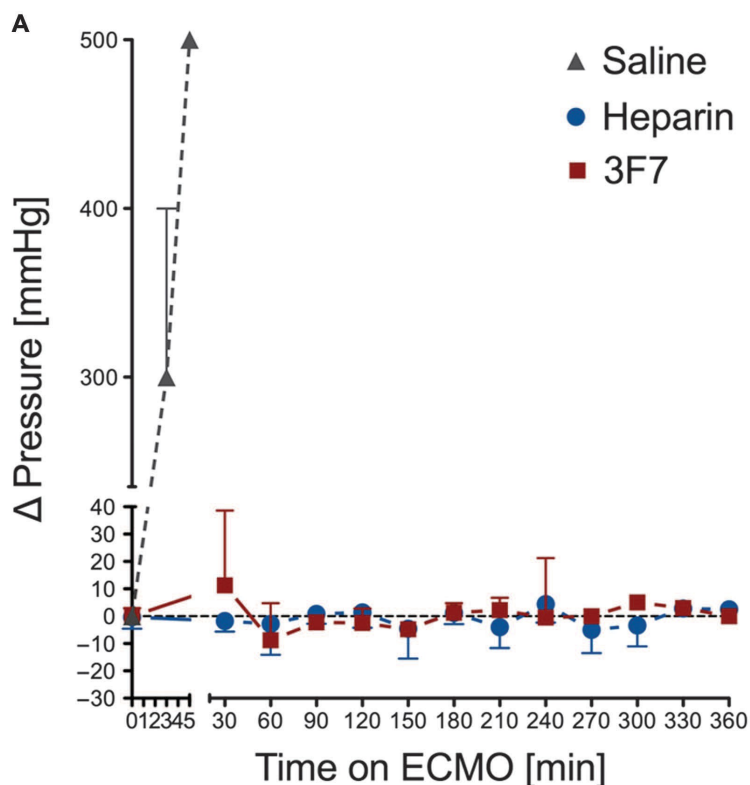
The main finding of the experiment was the reduction of central venous pressures ( $CVP_{sup}$  and  $CVP_{inf}$ ) with increasing venoarterial ECMO flow. Portal flow and systemic blood pressure were improved during increased right ventricular load. A decrease in venous pressure ( $CVP_{sup}$ ) was noted in the stage with normal right ventricular load, while the effect on systemic perfusion was less pronounced in this setting. Both Portal flow and Mean Arterial Pressure increased to normal or even supernormal levels, indicating unnecessary hypervolemia. In the clinical situation with ongoing hemorrhage, the fluid resuscitation would be limited according to the concept of permissive hypotension<sup>129</sup>. The ECMO cannulation technique used in this study may be useful for solving other surgical problems as well. Blood is drained from both the SVC and the IVC. Oxygenated and normothermic blood is infused in the aorta with a flow rate approaching normal cardiac output if required. Blood flow and pressure in the pulmonary circulation are reduced during VA ECMO<sup>130</sup>. This may limit bleeding from large pulmonary vessels and possibly decrease the need for pulmonary resections<sup>131</sup>. The IVC can be clamped without compromising venous return or causing venous congestion in the abdominal organs or lower extremities. An injury to the retrohepatic IVC or hepatic veins is lifethreatening to the patient and extremely difficult for the trauma surgeon. Combined with ECMO, total hepatic vascular exclusion (TVE) may be applied with preserved systemic perfusion<sup>132, 133</sup>. The short-term risks with ECMO include bleeding complications during cannulation, bleeding caused by tube disconnection, cannule displacement and air or clot embolization. These risks are reduced with the growing experience of a dedicated ECMO team. Short perfusion time in most trauma patients reduces the risk of infections and coagulation disorders. In this study systemic heparin was used due to economic reasons. In the clinical trauma situation however, surface heparinized circuits would be the equipment of choice.

## Study II

### Results study II

#### *Thromboprotection. The pressure gradient over the oxygenator*

In the control (saline) group no anticoagulation was used in either animals or ECMO system. Without anticoagulation the pressure gradient over the oxygenator rapidly increased to > 500 mmHg, the roller pump failed to maintain circulation, and within < 3 min, the extracorporeal circulation was completely occluded. In two other control rabbits it was impossible to drain blood into the ECMO system because of thrombotic occlusion of the cannulas. In contrast Heparin efficiently inhibited occlusion of the cardiopulmonary bypass system. The blood pressure over the oxygenator remained low (<15 mm Hg) throughout the 6-hour ECMO run. A single dose of 3F7 administered 5 min prior the start of ECMO provided similar thromboprotection to that observed with Heparin. In the 3F7 group the pressure gradient over the oxygenator also was < 15 mm Hg (Fig.15 A). Arterial oxygen saturation (SO<sub>2</sub>) reached 100% in both the heparin- and 3F7 groups and was stable throughout the 6-hour ECMO period indicating normally functioning oxygenators.

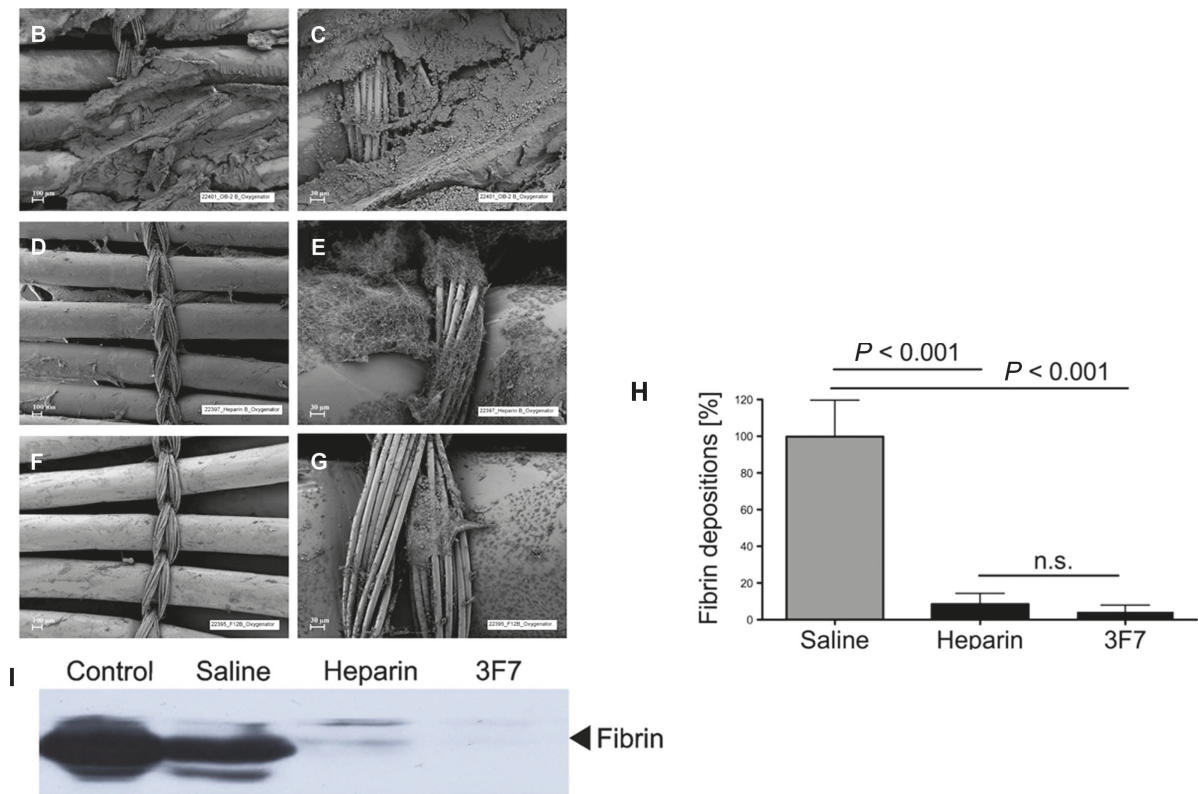


**Figure 15 A. Changes in the oxygenator pressure.**

The rabbits were pretreated with a single bolus of saline, heparin or 3F7, 5 min before the start of ECMO. The blood pressure gradient over the oxygenator was continuously monitored at the inflow and outlet by a pressure sensor.

### ***Thromboprotection. The fibrin deposition in the oxygenator***

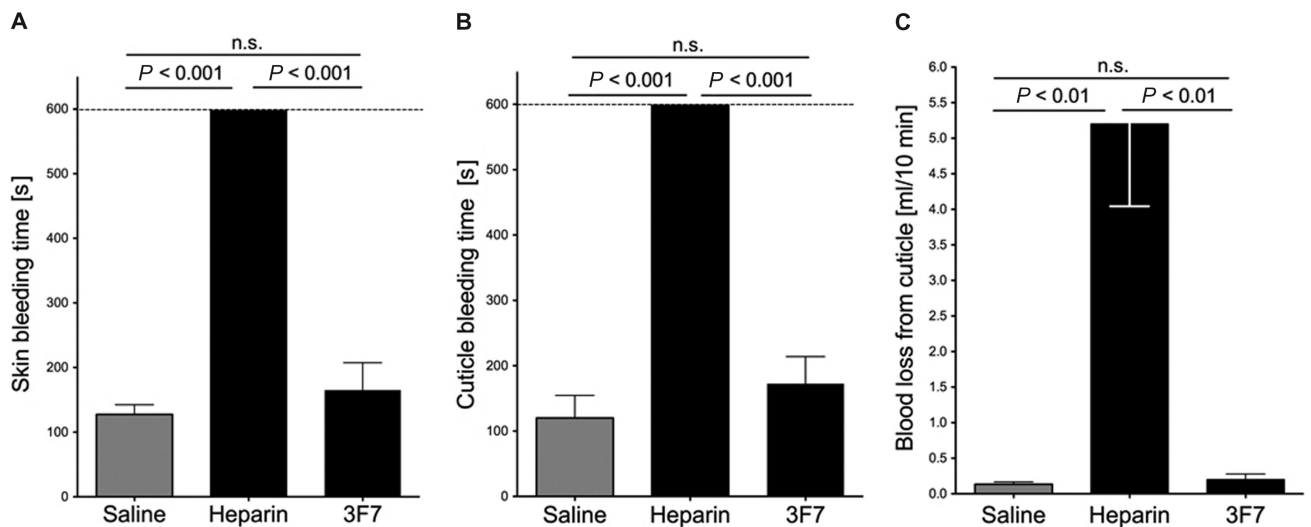
SEM examination of the opened oxygenators revealed large clots of fibrin and deposited blood cells in the Saline treated group (Fig.15 B-C) already after the first minutes of perfusion, whereas fibrin depositions after 6 hours were minimal in oxygenators of Heparin- and 3F7-treated rabbits (Fig. 15 D-G). When quantified in SEM it was found that the fibrin deposits were significantly reduced in both Heparin and 3F7-treated animals compared to saline controls ( $p < 0.005$ ) (Fig 15 H). Oxygenator extracted material was analyzed for fibrin deposition by Western blotting and consistent with the SEM images the fibrin signal was high in the saline treated group and largely reduced in the Heparin- and 3F7 anticoagulated systems (Fig.15 I).



**Figure 15 B-I.** Scanning Electron Microscopy of oxygenator capillaries (15B-G). Quantification of fibrin deposition per HPF (15H). Western blot analysis of fibrin signal from oxygenator clots (15 I).

## Bleeding in vivo

Heparin treatment largely prolonged incision-provoked skin bleeding time in rabbits from  $130 \pm 15$  to  $>600$  s (Fig. 16 A). Heparin also impaired hemostasis at wound sites induced by clipping the cuticle tip after 6 hours of extracorporeal bypass. Cuticle bleeding time was  $>600$  s and accompanied by a mean blood loss of  $5.1 \pm 1.1$  ml (Fig 16 B and C). In contrast, the hemostatic capacity of 3F7-treated rabbits was intact. 3F7-treated rabbits had similar mean bleeding times as saline-infused controls (skin:  $160 \pm 40$  s versus  $130 \pm 15$  s and cuticle:  $165 \pm 40$  s versus  $120 \pm 30$  s) (Fig. 16 A-B). Blood loss from cuticle wounds was also not significantly increased over that of saline-treated animals ( $0.2 \pm 0.05$  ml/10 min versus  $0.3 \pm 0.1$  ml/10 min) (16 C).



**Figure 16 A-C. Differential effects of 3F7 and heparin on hemostasis.** Skin bleeding time (A), Cuticle bleeding time (B) and Blood loss from cuticle (C).

## Discussion study II

Anticoagulant therapy is one of the most common forms of medical intervention and used for treatment and prevention of thrombosis. Bleeding is the primary complication of anticoagulant therapy and a significant risk of all currently used anticoagulants, even when maintained within their therapeutic ranges<sup>134, 135</sup>. Heparin is obligate as clot prevention in ECMO and bleeding is also the most frequent and severe complication of ECMO-treatment<sup>136</sup>. The present study shows a safe thromboprotective strategy based on inhibition of FXIIa activity in a clinically meaningful setting. The anti-FXIIa antibody 3F7 is as efficient as heparin for thromboprotection in an ECMO system, but without the heparin-associated impairment of hemostatic capacity. Humanized antibodies expressed in mammalian cells have been used clinically for many years and are an important class of human therapeutic products<sup>137</sup>. The fully human 3F7 antibody is expected to display minimal immunogenic potential, and the long serum half-life is ideal for prophylactic use. 3F7 has the necessary properties for further analysis as a mode of safe anticoagulation in clinical trials. Over the past 40 years, more than 69000 patients have been treated with ECMO, and the modified heart-lung machine has salvaged many lives by providing gas exchange and systemic perfusion. However, the ECMO circuit exposes blood to non-physiological surfaces that directly induce FXII contact activation leading to coagulation and complement-activated inflammation. Currently heparin is the standard anticoagulant in ECMO. Heparin increases bleeding, which is the principal cause of morbidity and mortality in ECMO therapy and stresses the need for safer anticoagulation. Although coating the gas-exchanging capillaries in oxygenators with heparin<sup>138</sup>, phosphorylcholine<sup>139</sup> or fibronectin<sup>140</sup> has improved the biocompatibility, there is a continuous FXIIa generation on bypass circuit surfaces<sup>141</sup>. Non-physiological shear and mechanical forces activates platelets in the bypass circulation. Activated platelets release the inorganic polymer Polyphosphate, which provides an alternative source of FXIIa generation with implications for fibrin production within the growing thrombus<sup>142</sup>. Nitric Oxide inhibits platelets and provides thromboprotection in experimental ECMO systems<sup>125, 143</sup>. In contrast, 3F7 interferes with both artificial surface- and platelet-produced procoagulant FXIIa activities.

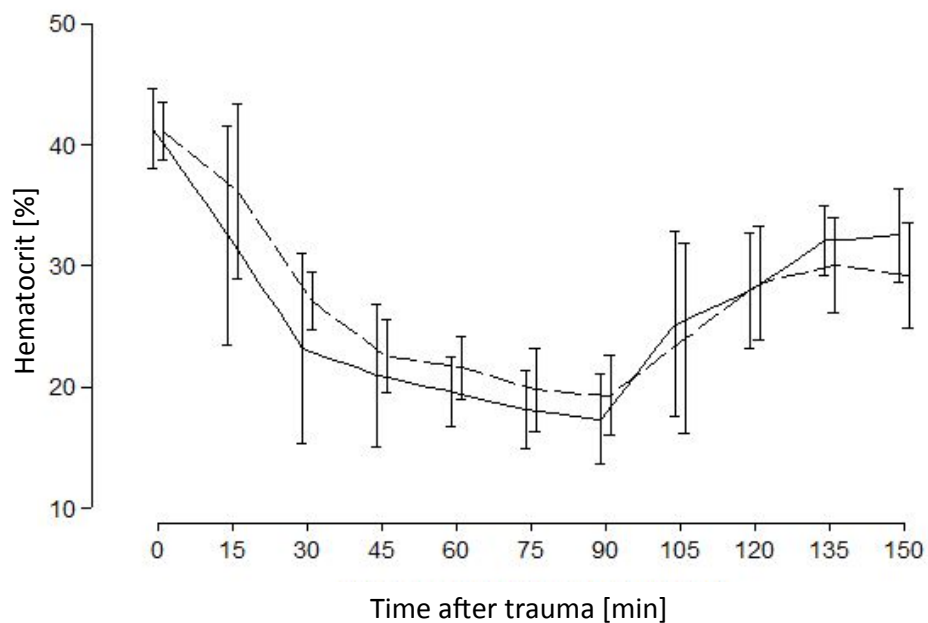


## Study III

### Results study III

#### The Hemorrhagic Phase

After traumatic injury and 90 minutes of hemorrhagic shock, all animals (n=10) were in a critical state and fulfilled the set criteria of hypotension (MAP<20 mmHg), hypothermia (T<32°), metabolic acidosis (pH<7.3) and coagulopathy. There was no significant difference in acidosis (BE, lactate or pH), temperature, hemodynamic status (HR, MAP) or ROTEM between the two groups after the 90 minutes of traumatic hemorrhage. The hematocrit did not differ between the two groups after hemorrhage and resuscitation ( $p=0.84$ ) indicating that the hemorrhage and transfusion protocols were equal (Fig.17)

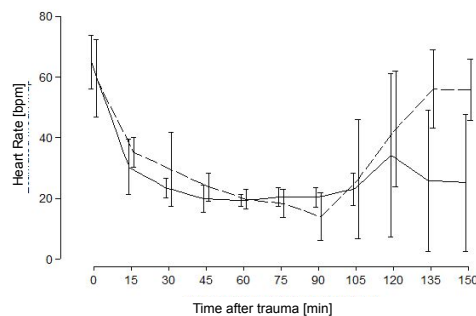


**Figure 17. The Hematocrit during the study.** The exsanguination and retransfusion in the two groups were equal. Control (—) ECMO (-----)

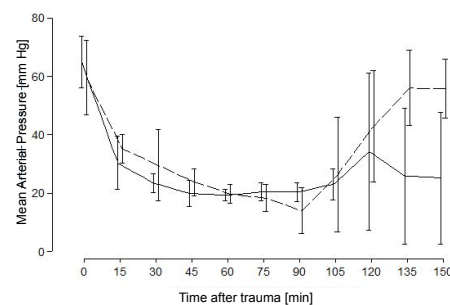
## The Resuscitation phase

### *Hemodynamic status*

Immediately after initiation of ECMO the HR and MAP improved and after 60 min the animals were back to a normal circulatory state. In the control group the HR kept falling after resuscitation and MAP increased during the first 30 min but never exceeded 40 mm Hg and then decreased again. There was a statistical significant difference, favoring the ECMO group, after 60 minutes of resuscitation both according to HR and MAP ( $p=0.01$  and  $p=0.01$  respectively) (Fig. 18 A-B).



18 A

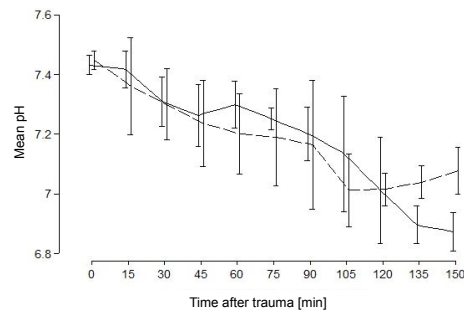
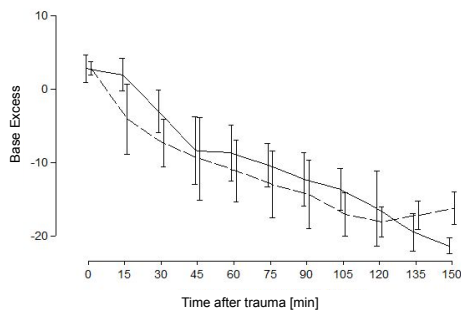


18 B

**Figure 18. The animals circulatory status during the study.** 90 minutes after hemorrhagic shock there was no difference in heart rate (Fig.18A) or mean arterial pressure (Fig. 18B) between the two groups but after 60 min of resuscitation the heart rate and mean arterial pressure in the ECMO group were significantly improved. ( $p=0.01$  respectively). Control (—) ECMO (---).

### *Acid-Base balance*

There was a significant rise in lactate ( $>8\text{mM}$ ), decrease in BE ( $-10$ ) and fall in pH ( $<7.3$ ) in both groups at the end of hemorrhagic shock phase. The lactate concentration slowly started to fall after initiation of ECMO but continued to rise in the control group ( $>12\text{mM}$ ). After the resuscitation phase for 60 min lactate was overall significantly lower in the ECMO group ( $p=0.01$ ). Base Excess started to increase after 30 minutes of ECMO treatment and was overall significantly improved compared to the control group ( $p=0.01$ ) (Fig. 19 A). After 20 min of ECMO, pH started to increase and was above 7.0 after 60 min. Meanwhile the pH continued to be low in the control rabbits ( $<6.9$ ) the pH after 60 min was significantly higher in the ECMO group ( $p=0.01$ ) (Fig.19B).



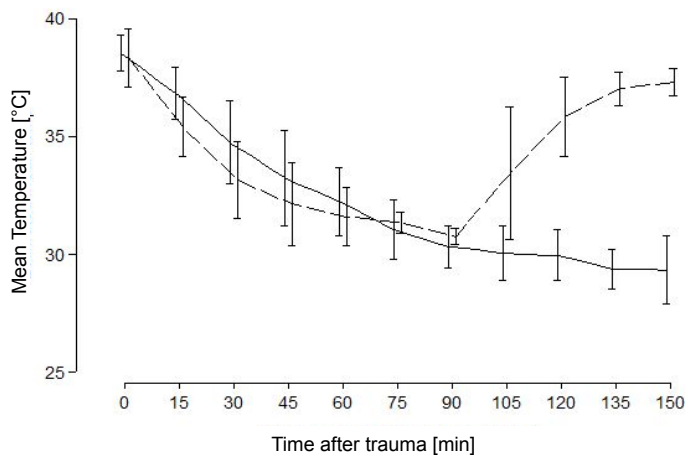
19 A

19 B

**Figure 19. The animal's acid-base balance during the study.** 90 minutes after hemorrhagic shock there was no difference in base excess (Fig. 19A) or pH (Fig.19B) between the two groups. After 60 min of resuscitation the base excess and the pH were significantly higher in the ECMO group ( $p=0.01$  respectively). Control (—) ECMO (----).

### Body temperature

Initiation of ECMO efficiently increased the animal's body temperature and after 60 minutes of active warming they had reached their initial temperature ( $>37^{\circ}\text{C}$ ), which was significantly improved, compared to the control group, where the body temperature remained low (below  $30^{\circ}\text{C}$ ) despite warm blood transfusion ( $p=0.01$ ) (Fig.20).



**Figure 20. The animals body temperature.** Initiation of ECMO increased the animal's body temperature efficiently and after 60 min the animals in the ECMO group were significantly warmer ( $>37^{\circ}\text{C}$ ) than the controls ( $p=0.01$ ). Control (—) ECMO (----).

### ***Coagulation In Vitro (Table 1)***

The contact pathway of coagulation was evaluated with aPTT and ROTEM Clotting Time, (CT<sub>Intem</sub>). The aPTT decreased in the ECMO group during the 60 min of resuscitation ( $17.7 \pm 2.7$  s –  $11.0 \pm 2.3$  s), while aPTT, in the control group, continued to rise ( $20.6 \pm 4.3$  s –  $29.0 \pm 8.7$  s). The difference between the two groups did not reach statistical significance ( $p=0.07$ ). CT<sub>Intem</sub> increased in both groups, but even though there was no significant difference at the end of resuscitation, the rise was higher in the control group, control:  $948 \pm 693$  s vs. ECMO:  $290 \pm 93$  s.

The tissue factor dependent extrinsic pathway of coagulation was analyzed by INR, ROTEM (CT<sub>Extm</sub>) and (CFT<sub>Extm</sub>). INR decreased after 60 min of ECMO ( $1.74 \pm 0.33$  -  $1.44 \pm 0.2$ ) while it remained on the same level in the control group ( $1.3 \pm 0.15$  -  $1.36 \pm 0.09$ ). The extem clotting time (CT<sub>Extm</sub>) was considerably reduced in the ECMO group ( $764 \pm 687$  -  $79 \pm 8$  s) while it was largely prolonged in the control group ( $69 \pm 4$  -  $764 \pm 687$ s) and there was a difference between the groups at the end of the experiment. Control clotting time was  $764 \pm 687$ s versus ECMO clotting time  $79 \pm 8$  s ( $p=0.17$ ).

The clot formation time (CFT<sub>Extm</sub>) followed the same pattern with a clear decrease in the ECMO group ( $1141 \pm 965$  -  $111 \pm 19$  s) and a clear increase in the control ( $170 \pm 14$ - $1770 \pm 1001$ s). The extrinsic clot formation time was at the end significantly lower in the ECMO group ( $p<0.01$ ).

Analyzing the clot firmness with ROTEM gave the following results. MCF<sub>Extm</sub> (reflecting both platelet and fibrinogen contribution) was slightly increased after ECMO for 60 min. ( $42.2 \pm 10.4$ mm -  $51.6 \pm 2.8$  mm) while it was reduced in the control ( $51.2 \pm 0.9$  mm-  $35.6 \pm 10.6$  mm). The difference was not significant ( $p=0.2$ ). The MCF<sub>Fibtem</sub> (only fibrinogen contribution) showed minimal detectable values at the end of the experiments equally in both groups. The Fibrinogen levels were also equally diminished in the two groups and did not increase after transfusion.

Neither in the study group nor in the control group the ROTEM curves showed any fibrinolysis. Analyses with HEPTTEM, blocking heparin effect, did not change the INTEM CT or CFT variables, confirming that there was no heparin effect in the system.

The platelet count was equal in the two groups initially but after 90 min of hemorrhagic shock the platelet count was lower in the ECMO group. The number of circulating platelets kept falling and was at the end of the resuscitation phase significantly reduced in the ECMO group ( $85 \pm 17$ ) compared to the control group ( $149 \pm 20$ ) ( $p=0.02$ ).

	<b>Baseline</b> <b>0 min</b>	<b>Control</b> <b>90min</b>	<b>ECMO</b> <b>90 min</b>	<b>P<sup>1</sup></b>	<b>Control</b> <b>150 min</b>	<b>ECMO</b> <b>150min</b>	<b>P<sup>2</sup></b>
<b>Temp (°C)</b>	38.4 ± 0.1	30.3 ± 0.4	30.7 ± 0.2	0.34	29.3 ± 0.4	37.3 ± 0.3	0.01
<b>pH</b>	7.44 ± 0.01	7.20 ± 0.04	7.16 ± 0.10	0.38	6.90 ± 0.03	7.08 ± 0.04	0.01
<b>Lactate (mM)</b>	1.07 ± 0.05	11.6 ± 1.4	8.9 ± 1.1	0.10	14.3 ± 1.1	7.8 ± 0.7	0.01
<b>BE (mM)</b>	2.79 ± 0.03	-12.3 ± 1.8	-14.3 ± 2.3	0.26	-21.4 ± 0.5	-16.2 ± 1.0	0.01
<b>Hb (g/L)</b>	134 ± 3	56 ± 4	62 ± 3	0.17	90 ± 16	93 ± 8	0.45
<b>PLT x 10<sup>9</sup>/L</b>	193 ± 17	131 ± 23	93 ± 18	0.17	149 ± 20	85 ± 17	0.02
<b>aPTT (s)</b>	14.4 ± 2.6	20.6 ± 4.3	17.7 ± 2.7	0.16	29.0 ± 87	11.0 ± 23	0.07
<b>INR</b>	0.9 ± 0.0	1.3 ± 0.15	1.74 ± 0.33	0.20	1.36 ± 0.09	1.44 ± 0.2	0.42
<b>Fibrinogen</b>	2.2 ± 0.0	1.2 ± 0.3	0.9 ± 0.2	0.38	1.3 ± 0.2	1.0 ± 0.2	0.38
<b>CT<sub>Ext</sub> (s)</b>	45 ± 2	69 ± 4	764 ± 687	0.04	827 ± 710	79 ± 8	0.17
<b>CFT<sub>Ext</sub> (s)</b>	42 ± 1	170 ± 14	1141 ± 965	0.46	1770 ± 1001	111 ± 19	0.01
<b>MCF<sub>Ext</sub> (mm)</b>	66.7 ± 0.5	51.2 ± 0.9	42.2 ± 10.4	0.30	35.6 ± 10.6	51.6 ± 2.8	0.20
<b>CT<sub>Int</sub> (s)</b>	181 ± 28	262 ± 357	245 ± 64	0.46	948 ± 693	290 ± 93	0.34
<b>MCF<sub>Fib</sub> (mm)</b>	11.3 ± 0.5	4.0 ± 0.3	4.2 ± 1.0	0.33	3.4 ± 1.0	4.8 ± 1.0	0.19
<b>EBT (s)</b>	66.0 ± 9.0	-	-	-	162 ± 21	55 ± 7	0.01
<b>CBT (s)</b>	70.5 ± 10.5	-	-	-	174 ± 15	75 ± 13	0.01
<b>Coagulopathy*</b>	No	++	+++		++	+	

**Table 1. Blood and coagulation.** Baseline represents all animals. The control and ECMO groups are compared after hemorrhagic shock (90 min) and after resuscitation (150 min) and p-values are calculated with Rank Sum Test. **P<sup>1</sup>**: 90 min **P<sup>2</sup>**: 150 min Values presented as Means with SEM. \*) Coagulopathy defined as aPTT > 26s

### ***Coagulation In Vivo (Table 1.)***

The baseline bleeding times according to Ear Bleeding Time (EBT) and Cuticle Bleeding Time (CBT) were equal in the two groups. After resuscitation the EBT was increased to  $162 \pm 21$  and the CBT to  $174 \pm 15$  s in the control group. In contrast, the hemostatic capacity of ECMO-treated rabbits was improved with an EBT of  $55 \pm 7$ s and a Cuticle mean bleeding time of  $75 \pm 13$  s. Both the difference in EBT ( $p=0.01$ ) and CBT ( $p=0.01$ ) were statistically significant, favoring the ECMO group.

### ***Outcome and Survival***

In the ECMO group three animals (3/5) were asystolic and one was severely bradycardic ( $HR < 10$ ) at cannulation. They all received external cardiac compressions and recovered with recapture of sinus rhythm and normalized blood pressure after transfusion and initiation of extracorporeal circulation. In summary all animals (5/5) in the ECMO group survived with stabilized vital status and improved coagulation. Two animals (2/5) in the control group were asystolic, 28 min respectively 30 min after initiation of resuscitation (transfusions were completed in both cases). They received external cardiac compressions but did not regain sinus rhythm. The surviving animals in the control group (3/5) were all in a deranged circulatory status.

### Discussion study III

Trauma-induced coagulopathy is an important reason for death in trauma victims<sup>78</sup>. Tissue injury and systemic hypoperfusion are needed for the development of acute traumatic coagulopathy<sup>64</sup> (ATC). When fully developed, coagulopathy is very difficult to reverse. This study shows that VA-ECMO improves acidosis and temperature in a traumatic experimental hemorrhagic model. The animals were efficiently rewarmed ( $>37^{\circ}\text{C}$ ) and could reach a pH above 7.0 within 60 min. ECMO also improved central circulation in terms of blood pressure and heart rate. When supported by VA-ECMO all animals in severe hemorrhagic shock survived. The extrinsic coagulation pathway reflected by clot formation time ( $\text{CFT}_{\text{Extm}}$ ) was improved and there was a trend of improvement in the intrinsic pathway as well. These findings were verified with skin and cuticle bleeding times.

To our knowledge no similar study has been performed before. We have used the rabbit-ECMO model before when evaluating the Factor XII antibody 3F7<sup>144</sup>. ECMO has previously been used in a rabbit-trauma model where ECMO-resuscitation for prolonged hemorrhagic shock (3h) improved tissue perfusion, reduced systemic inflammation and alleviated the intestinal damage that may be one important factor for multi organ failure (MOF)<sup>145</sup>. Park *et al* showed that rats exposed to both hemorrhagic shock and hypothermia have a prolonged clot formation time and reduced MCF<sup>146</sup>. The former finding is verified by the present results, but ECMO only slightly improved the clots firmness. This may be due to the consumption of fibrinogen. The fibrinogen levels were equally reduced after hemorrhagic shock in both groups and the ECMO system did not seem to aggravate fibrinogen consumption. Apart from a lower platelet count and much higher  $\text{CT}_{\text{Extm}}$  and  $\text{CFT}_{\text{Extm}}$  in the ECMO group after hemorrhagic shock there was no difference between the groups before resuscitation.

In spite of the initial advanced coagulopathy, several coagulation parameters improved after ECMO-resuscitation. Engstrom *et al* showed the importance of an increased pH and reduced lactic acidosis to avoid impairment of the coagulation cascade in two previous studies<sup>147, 148</sup>. We believe that the improvement of tissue perfusion with ECMO resulted in a gradual normalization of lactate and pH. This may have had a beneficial effect on the coagulation capacity. To gain efficiently strong clots after this type of trauma it may be necessary to substitute fibrinogen. In the present study the fibrinogen levels were consumed, stayed on a low level and was not substituted. This was verified by a reduction of the maximum clot firmness in both  $\text{MCF}_{\text{Extm}}$  and  $\text{MCF}_{\text{Fibtem}}$ . These findings suggest that the reduction was due mainly to low fibrinogen levels. In a clinical setting fibrinogen would have been substituted. Extracorporeal devices activates the coagulation system<sup>149</sup> and previous studies have shown decreased numbers of circulating platelets in extracorporeal devices<sup>150, 151</sup>. This finding was confirmed in the present study. The number of circulating platelets did not reach levels below  $50 \times 10^9/\text{L}$  and the clotting ability did not seem to be negatively affected. In vivo testing of the coagulation capacity with ear bleeding and cuticle bleeding times showed an improvement in the ECMO group.

ECMO in trauma has been somewhat controversial but has in recent years gained in popularity both in the post trauma ARDS situation and in hypovolemic shock during the early resuscitation phase<sup>101</sup>. In our clinical experience, we have successfully resuscitated selected patients with respiratory failure and concomitant hemorrhagic shock due to thoraco-abdominal trauma with venoarterial ECMO (non published data). An earlier experimental study described how VA-ECMO reduced systemic venous pressure while maintaining or improving systemic perfusion in both a normal circulatory state and in the setting of increased right ventricular load associated with acute lung injury<sup>152</sup>. ECMO may theoretically be a useful tool in reducing blood loss during major venous hemorrhage, but other positive or negative effects of ECMO in trauma need to be investigated. There have been concerns about using heparin in trauma patients on ECMO. We did not use systemic heparin as anticoagulant for the ECMO system but heparinized surfaces of tubings and oxygenators. Viscoelastic heparin test (HEPTTEM) did not show any increase of free heparin in the system. There was a trend of improved aPTT in the ECMO group and there were no problems with clotting in the circuit. In the future it may be possible to use anticoagulant drugs that do not affect the bleeding risk<sup>144</sup>.

The effects of ischemia-reperfusion in our study were not controlled but these effects were assumed to be equal in both groups. Inflammation and coagulation are largely activated in ECMO-systems<sup>153</sup>. The aim was to study the effect of ECMO support in the first initial 60 min of trauma resuscitation. The ability of ECMO to fast increase the body temperature during this time is obvious but the reduction of lactate and improvement of pH is somewhat slower. If ECMO had been extended for more than 60 minutes, maybe even continued for the first 24 post-trauma hours, the acid-base balance might have been improved further. A suboptimal ECMO flow, 100ml/ min instead of 300ml/ min, was used because of the limited amount of blood to prime the ECMO circuits. In clinical trauma scenarios there may be a continuing bleeding problem and difficulties to withhold a full flow. However, the ECMO circuit can be filled with as much blood as required, and the large bore cannulas are optimal for high volume infusion. This study was limited of some technical factors including the preparation of tracheostomy and laparotomy before the trauma and the priming of the ECMO circuit before the actual experiment started. The coagulation system in rabbits is slightly different compared to humans. The rabbit coagulation system is constructed as the human with extrinsic, intrinsic and a common pathway's and consists of the same coagulation factors. The serine proteases activity differs though. Rabbits have a 50 times higher activity of factor V than humans and the activity of factor XI and factor XIII are also slightly higher. This may explain why rabbits have shorter aPTT and PT<sup>154</sup>. The different activity may limit implications for human management and the variations of our coagulation data may be explained by a limited time of ECMO-resuscitation and a rather small number of study animals. However, this is the first experimental study showing ECMO's effect on temperature, acidosis and coagulation in an animal model of trauma and hemorrhage. Further studies to evaluate ECMO's role in trauma are obviously needed and indications for venoarterial ECMO in severe trauma need to be established in collaboration between the trauma- and ECMO communities.

In conclusion, VA-ECMO seems beneficial in an experimental traumatic hemorrhagic shock model for stabilization of central hemodynamics, improvement of acidosis, body temperature and coagulopathy. ECMO may also increase the likelihood of survival.

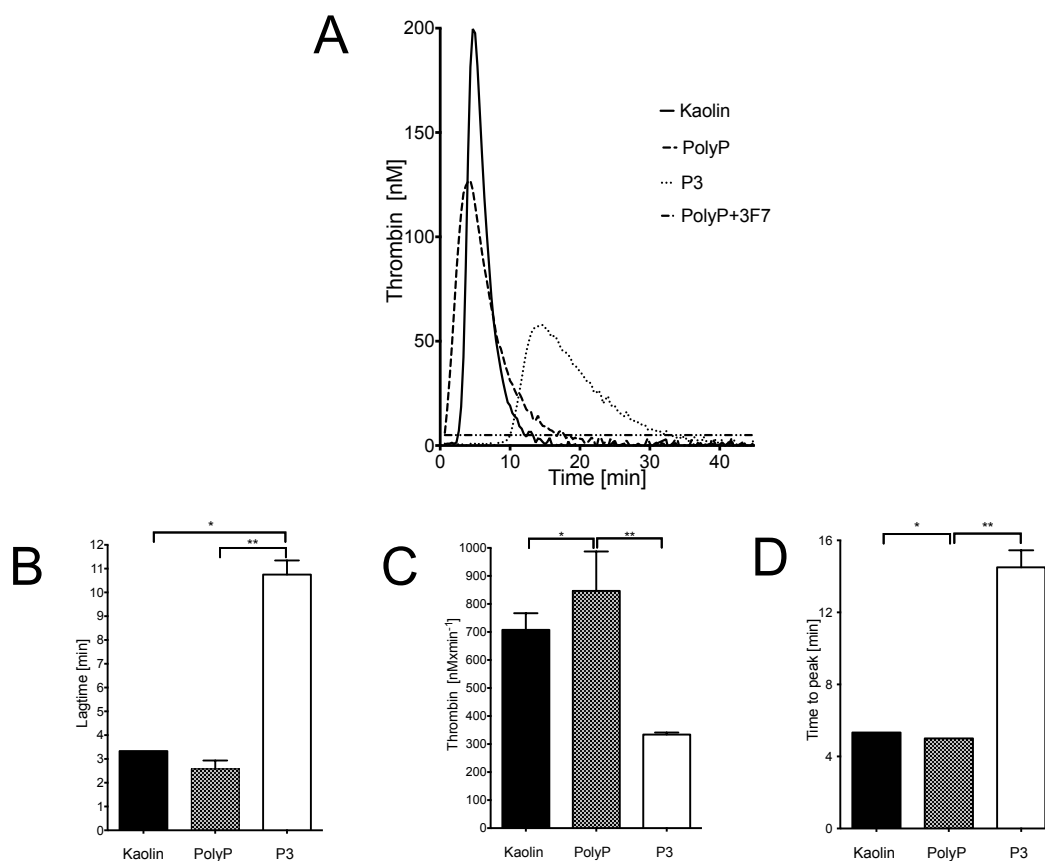


## Study IV

### Results study IV

#### *Polyphosphate enhances thrombin generation in a FXII dependent manner*

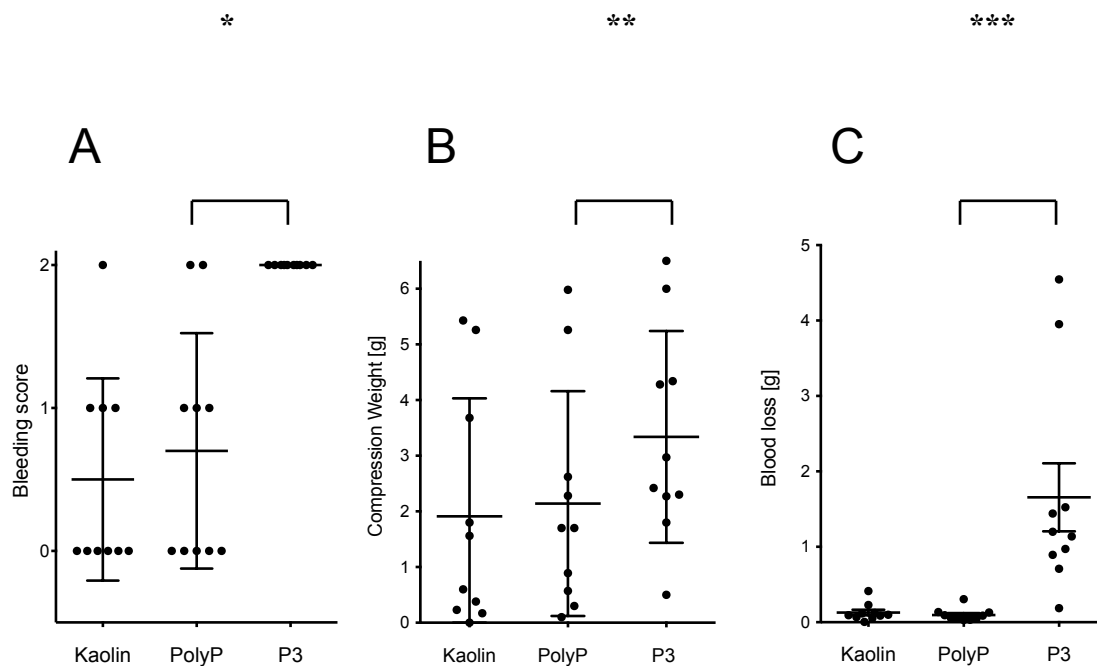
PolyP was able to enhance thrombin generation in plasma. PolyP was equally efficient as Kaolin and significantly better than P3 (Fig. 21A). The lag time to thrombin generation was  $2.58 \pm 0.25$  min for PolyP and  $10.75 \pm 0.42$  min for P3 and was significantly shorter ( $p < 0.004$ ). The time to thrombin peak was also shorter for PolyP  $5.00 \pm 0.01$  min compared to  $14.50 \pm 0.67$  min for P3 ( $p < 0.005$ ). The amount of generated thrombin was significantly greater when PolyP ( $846.5 \pm 99.5$  nmol) was compared to P3 ( $334.0 \pm 5.0$ ) ( $p = 0.035$ ). There was no difference when PolyP was compared to Kaolin. (Fig. 21 B-D). 3F7, the FXIIa inhibiting antibody 3F7 completely abolished PolyPs thrombin potential. Values are expressed as Mean  $\pm$  SEM.



**Figure 21. Real Time Thrombin Formation:** PolyP was equally efficient as Kaolin to induce thrombin generation (Fig.21 A) The lag time to thrombin generation (Fig.21B) and the time to peak (Fig.21D) was significantly shorter with PolyP compared to the control P3 ( $p < 0.004^{**}$ ) Fig.21B and respectively ( $p < 0.005^{**}$ ) (Fig.21D). The amount of generated thrombin was also significantly increased with PolyP compared to P3 ( $p = 0.035^{**}$ ) (Fig 21C). There was no difference when PolyP was compared to Kaolin according to thrombin generation time or amount (Fig.1B-B\*). 3F7, the FXIIa inhibiting antibody totally abolished polyPs thrombin potential, indicating that polyP drives thrombin formation by activating FXII (Fig.21A).

### ***Polyphosphate is hemostatic in liver injury***

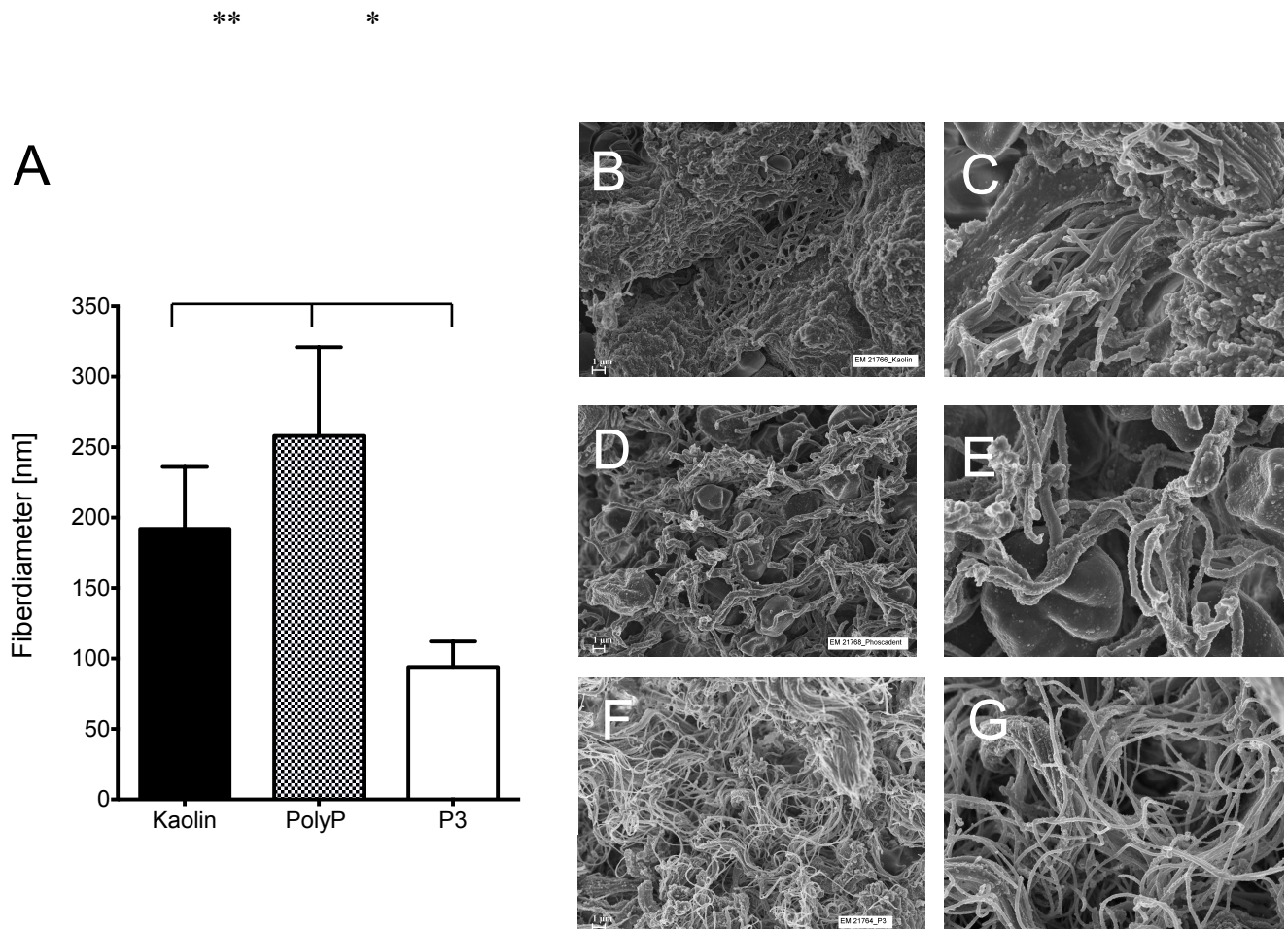
Visually PolyP had a better coagulative effect than P3 [ $0.7 \pm 0.2$  versus  $2.0 \pm 0.0$ ] ( $p=0.0007^*$ ). The visual bleeding in PolyP-treated wounds was comparable with that of Kaolin treated wounds [ $0.5 \pm 0.2$ ] (Fig 22A). Without reaching significant difference, there was a trend that the compress material from PolyP and Kaolin treated wounds were lighter compared with P3\*\* [ $2.1 \pm 0.6$  g,  $1.9 \pm 0.7$  g and  $3.3 \pm 0.6$  g (P3)] (Fig 22B). When blood loss was calculated through hemoglobin amount in compress material, PolyP-treated wounds bleed significantly less ( $p<0.0002^{***}$ ) than control (P3) [ $0.1 \pm 0.03$  g versus  $1.66 \pm 0.45$  g]. The blood loss from the PolyP treated wounds was comparable to the Kaolin-treated wounds [ $0.1 \pm 0.03$  g versus  $0.1 \pm 0.04$  g] (Fig 22 C).



**Figure 22. Polyphosphate is hemostatic in liver injury.** A: Visual Bleeding Score (0-2), B. Blood loss according to compression weight (g), C. Calculated blood loss by measured hemoglobin (g)

### ***Polyphosphate enhances fibrin clot structure at the wound site***

Scanning Electron Microscopy (SEM) showed an irregular non-organized structure of fibrin fibrils in Kaolin treated wounds. P3-treated wounds had thin loosely connected fibrils, whereas PolyP-treated wounds had fibrils that were thick and gave a reinforced impression. Fibrin clots in the presence of PolyP, revealed thicker fibrils than clots made without PolyP. The fibrin fibrils in PolyP-treated wounds were significantly thicker than both control- and Kaolin-treated wounds, ( $p < 0.0001^*$  and  $p < 0.0001^{**}$  respectively) [ $258 \pm 63\text{nm}$ ,  $94 \pm 18\text{nm}$  and  $192 \pm 44 \text{ nm}$ ] (Fig. 23 A-G).

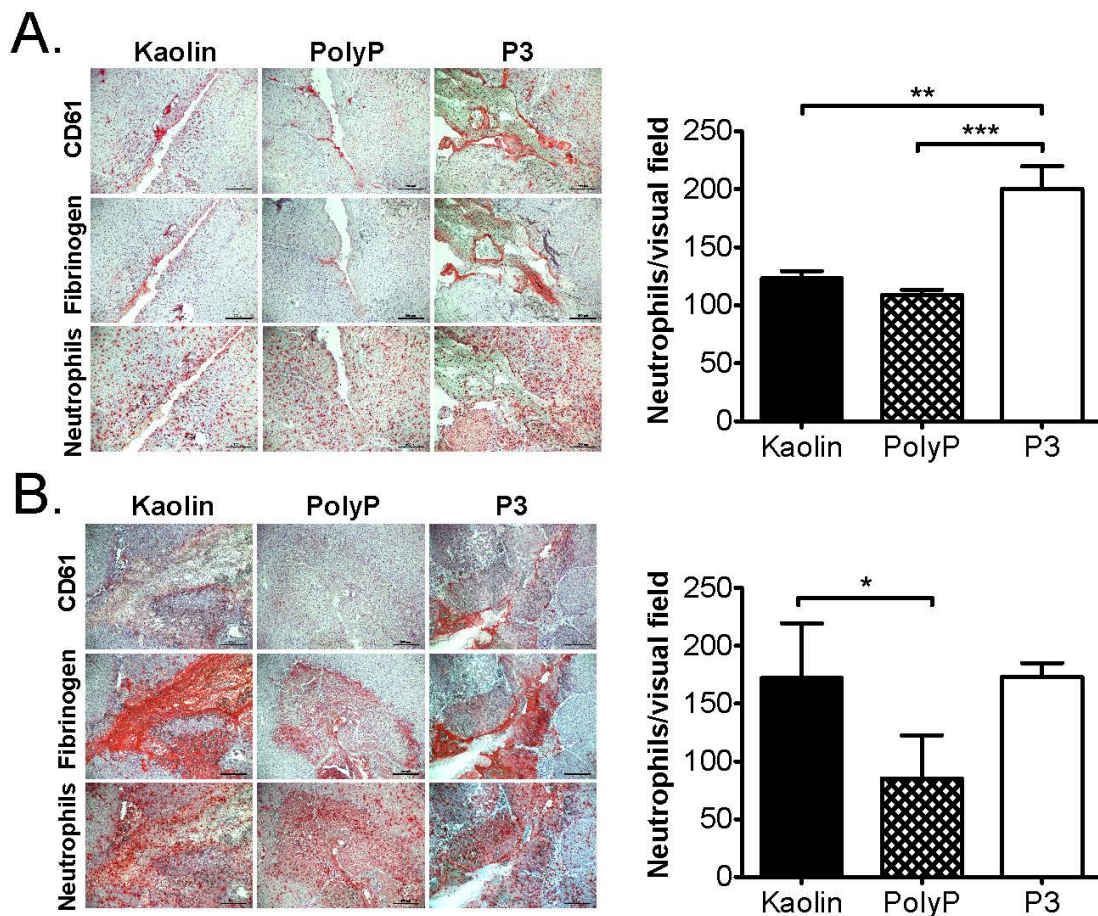


**Figure 23. SEM examination of fibrin clots from liver wounds:** Polyphosphate enhances the fibrin clot structure. Fibrin fibrils in Kaolin treated wounds showed an irregular non-organized structure (Fig.23B-C), PolyP-treated wounds showed fibrils that were thick and gave a reinforced impression (Fig.23D-E). P3-treated wounds had thin loosely connected fibrils (Fig.23F-G). The fibrin fibrils in polyP-treated wounds were significantly thicker than P3 treated wounds ( $p < 0.0001^*$ ) (Fig.23A).

### *Polyphosphate has minor inflammatory effects at the wound site*

30 min after the liver injuries were treated the number of infiltrating neutrophils were significantly higher in the wounds treated with P3 compared to the wounds treated with PolyP and Kaolin [ $201\pm25$ ,  $109\pm13$  and  $123\pm22$ ] ( $p<0.0001$ ) (Fig. 24 A\*\* and \*\*\*).

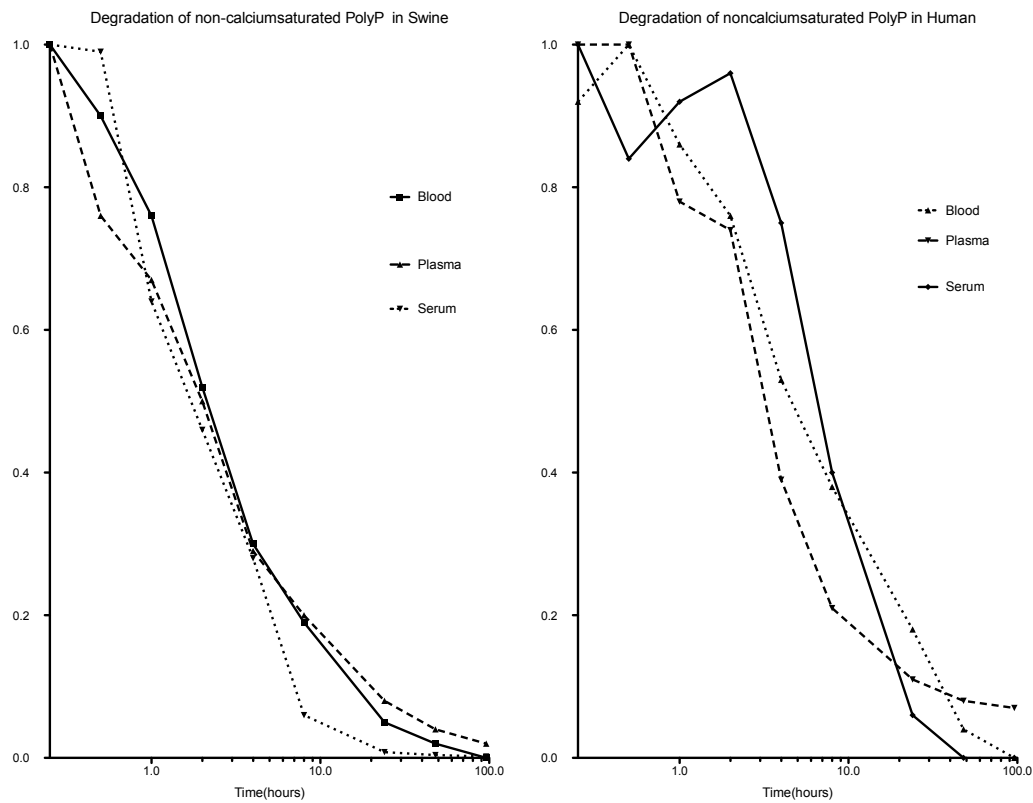
4 h after treatment of the liver injuries the number of infiltrating neutrophils was significantly lower in the PolyP-treated wounds compared to both Kaolin-treated (\*) and controls [ $85\pm37$ ,  $172\pm47$  and  $173\pm12$ ] ( $p<0.0065$ ). In Kaolin-treated wounds the neutrophil level was significantly increased 4 hours compared to 30 min after treatment [ $172\pm47$  versus  $123\pm22$ ] ( $p<0.01$ ). At this time the liver cells were more separated in both Kaolin- and PolyP- treated wounds Fig (24 B).



**Figure 24. Polyphosphate has minor inflammatory effects at the wound site.** Immunohistochemistry 30 min (Fig. 24A) and 4 h after treated liver injuries (Fig. 24B). 30 min after the liver injuries were treated the number of infiltrating neutrophils were significantly higher in the wounds treated with P3 compared to the wounds treated with polyP and Kaolin ( $p<0.0001$  respectively) (Fig. 24A \*\* and \*\*\*). 4 h after treated liver injuries the number of infiltrating neutrophils was significantly lower in the polyP-treated wounds compared to both Kaolin-treated (\*) and controls [ $85\pm37$ ,  $172\pm47$  and  $173\pm12$ ] ( $p<0.0065$ ).

### ***Polyphosphate is degraded in blood, plasma and serum***

PolyP was stable in water and PBS for at least 16 days. PolyP was degraded at an equal rate in blood, plasma and serum. PolyP had a half-life of 2.5 h in swine plasma (Fig. 25A) and 4 h in human plasma (Fig. 25B). After 4 days it was not traceable any more.



**25 A**

**25 B**

**Figure 25. Degradation of long-chained PolyP.** Swine Blood, Plasma and Serum (25A). Human Blood, Plasma and Serum (25B).

## Discussion study IV

The principle of creating hemostasis with biochemical agents is far from new. Hemostatic Agents (HA) have been used by several ancient cultures. The Egyptians treated bleedings with topical applications of raw meat as early as 1600 B.C.<sup>155</sup> and at the time of Hippocrates (460-377 B.C.) copper sulfate was used to induce artificial hemostasis. Today we know more about the mechanisms of coagulation and we still use the same principals as our ancestors did. What hemostatic mechanism, the Egyptian meat was driving remains unknown. One might speculate if the external provided Tissue Factor (TF) might have induced the TF: FVIIa coagulation pathway. However it is known that  $\text{Cu}^{2+}$  binds to FXII and stimulates contact activation, driving FXII mediated coagulation<sup>156</sup>. Soft white china clay is termed Kaolin and has also been used since thousands of years as HA. The chief constituent of Kaolin (as in all clays) is hydro-alumina-silicate (HAS). The negatively charged molecule of HAS activated FXII, which induces coagulation. Kaolin forms the basis of the activated partial thromboplastin time (aPTT), an assay universally accepted as a measure of “hemostatic competence” and the effectiveness of the intrinsic pathway.

In modern clinical medicine different agents in several ways like hemostatic field dressings, topical agents and systemically administered hemostatic agents may improve hemostasis. Kaolin is FDA approved as HA in hemostatic field dressings (e.g. Quick-Clot<sup>®</sup> and Combat<sup>®</sup> gauze). These dressings are well used in civilian prehospital care and conflicts of war<sup>157</sup> and has been shown to efficiently induce clotting<sup>158-160</sup>. A disadvantage is that Kaolin causes an exothermic reaction and pain. Animal studies and a case series of bleeding trauma patients have shown thermal injuries, foreign body reactions and fibrosis<sup>161, 162</sup>. Another silicate based hemostatic dressing (WoundStat<sup>®</sup>) was withdrawn from the market because it caused severe endothelial injury and significant trans mural vessel damage. The granules wandered in the venous circulation and got trapped in the lung with associated thrombosis<sup>163</sup>. To summarize, silicates are efficient in inducing the contact pathway of coagulation but their side effects can be severe. Novel hemostatic dressings with chitosan (polymers of N-acetyl-glucosamine) e.g. TraumaStat<sup>®</sup>, HemCon<sup>®</sup> and Celox<sup>®</sup><sup>164</sup> are vasoconstrictors that adhere to tissues, accelerates the concentration of platelets and red blood cells. Chitosan also to activates FXII<sup>165</sup> but is not biodegradable. Topical Agents (TA) is used in the operating theatre when controls of bleeding by standard surgical techniques (suture, ligature or cautery) are ineffective or impractical. Thrombin with gelatin (FloSeal<sup>®</sup>, Surgiflow<sup>®</sup>) converts the patient’s fibrinogen to fibrin. The gelatin matrix provides scaffolding for clot formation and swelling for tamponade effect<sup>166, 167</sup>. A disadvantage with gelatin bound thrombin is that the swelling can cause significant compression. Fibrin sealants (TISSEEL, Evicel<sup>®</sup>) are component mixtures of concentrated human fibrinogen, bovine thrombin and calcium, combined by an applicator to form a fibrin clot. These are excellent hemostatic devices not depending on patients clotting but require time for preparation and are expensive. Fibrin sealant patches (TachoSil<sup>®</sup>) are biodegradable collagen matrix coated with human fibrinogen and thrombin. When in contact with physiologic fluid the coating dissolves and diffuses into the tissue and a fibrin clot is formed<sup>168 169</sup>.

Recombinant FVII (rFVIIa), a systemically administered HA, was developed as a treatment for hemophilia patients with antibodies against FVIII and FIX in the 1980s. Its use in the management of traumatic hemorrhage was first described in 1999<sup>170</sup> and it has been used in both civilian and military trauma since then<sup>171, 172</sup>. In theory rFVIIa only induces clot formation where there is exposure of TF and systemically administered rVIIa should work at the local wound site. rFVIIa use has been the subject of randomized controlled trials in trauma patients and no benefit in terms of survival has been shown in these studies, although there may be a potential benefit in reduction of blood product transfusion and a reduction in the incidence of Acute Respiratory Distress Syndrome (ARDS)<sup>38, 173</sup>. The use of rFVIIa in combat medical treatment has declined significantly since early 2010<sup>174</sup>. Damage Control Resuscitation with targeted blood product replacement and the use of bedside thromboelastometry is one likely explanation of this trend<sup>175</sup>. The recognition of arterial thrombosis in connection with rFVIIa treatment is another<sup>176</sup>.

In the present study the liver was chosen as experimental model, to analyze the hemostatic capacity of PolyP, because it has extensively been used as a bleeding-model in previous research and hemorrhage from the liver-parenchyma is difficult to control<sup>177, 178</sup>. Synthetic produced PolyP of similar chain length as in platelets was used to activate the intrinsic pathway of coagulation. PolyP efficiently caused hemostasis in grade II liver injuries. The finding that a FXII-inhibiting antibody could block PolyP's thrombin potential indicates that PolyP drives thrombin formation by activating FXII. The intrinsic part of the coagulation cascade, which adverse effect can induce pathologic thrombus formation, was in this study used for something positive, to create local hemostasis<sup>179, 180</sup>. One of the desired properties of HAs is biodegradability to avoid foreign body reactions and bacterial overgrowth. PolyP was degraded to an equal rate in blood, plasma and serum but was stable in water and PBS. It can therefore be concluded that PolyP is degraded independently of cellular factors, but through enzymes present in plasma and serum. Phosphatases normally turn proteins off by de-phosphorylation. One of the most common phosphatases in humans is alkaline phosphatase (ALP). The enzyme is also detectable in blood and originates predominantly from liver and bone<sup>181, 182</sup>. It seems reasonable to assume that this enzyme is involved in the degradation of PolyP. The degradation-pattern of PolyP was the same in swine and human blood and these findings confirm previous reports on the lability of PolyP<sup>180, 183</sup>. PolyP enhanced the thrombin formation, shortened the time to clot formation and increased the clot strength. In vivo it had a clear coagulative effect on the bleeding liver injuries and the effect was comparable to the effect of Kaolin. It was obvious that the weight of the compression material to a large extent was influenced by abdominal fluid. To calculate the true bleeding, the hemoglobin amount in each compress was analyzed. SEM also revealed that PolyP stimulated to synthesis of thicker fibrin fibrils than both the control and Kaolin. This effect on fibrin fibrils has been documented before<sup>184, 185</sup>. The fact that PolyP generates thicker fibers than Kaolin might reflect that it acts on several levels during fibrin-production whereas Kaolin exclusively activates FXII. PolyP did not induce the same inflammatory reaction on the liver tissue, as did Kaolin. Both Kaolin-treated and PolyP-treated wounds showed signs of edema (increased interstitial space). This may be an effect of FXII induced Bradykinin-formation<sup>186</sup>.

The PolyP we used did not optimally adhere to the liver wounds. If PolyP should be taken further in the process of developing a new hemostatic agent it needs to be manufactured in a way that it adheres better to bleeding surfaces. As is the case with other HAs, PolyP probably needs to be coupled to some sort of biodegradable matrix. Another option of using PolyP with potent coagulation activity may be to impregnate suture material with the substance, minimizing blood loss from suture sites. PolyP shall only be used locally and not be injected intravenously since it can cause pulmonary embolism<sup>142</sup>.

In summary the present study shows that polyphosphate is effective in initiating coagulation and fibrin synthesis in actively bleeding liver wounds. It is degradable in swine and humans and it does not cause thermal injuries or inflammation to the extent that Kaolin containing hemostatic agents does. The possibility of producing large quantities of polyphosphate at low costs could make this an interesting hemostatic device for the future.



## **5. CONCLUSIONS AND FUTURE PERSPECTIVES**

### **Conclusions**

Based on Studies I-IV in this thesis the following conclusions were reached:

- I. Venoarterial ECMO reduces central venous pressure while maintaining or improving systemic perfusion.
- II. Targeting FXIIa provides safe anticoagulation in ECMO by inhibiting thrombus formation with intact hemostasis.
- III. Venoarterial ECMO improves coagulopathy after hemorrhagic shock.
- IV. Polyphosphates induces hemostatic coagulation in liver injuries, with minor inflammation.

### **Future Perspectives**

The care of trauma patients has improved significantly during the last decades. Despite this, thousands of people die everyday because of severe trauma and sometimes insufficient care. In the developing countries many lives can be saved by improvement of the transport systems, the prehospital care and the standard of the receiving hospitals. In the industrialized world, safety programmes, efficient prehospital care, well structured trauma triage and damage control surgery have strongly reduced the number of patients dying after injury. However, there are still patients dying of hemorrhage and the connected complications. The results presented in this Thesis may hopefully contribute to the salvage of patients that earlier has met a wasted death after trauma. In the best of worlds our results may also support the development of new and safer anticoagulants and thereby reduce the number of patients with serious anticoagulant related bleedings. Let us hope so.



## 6. ABBREVIATIONS AND DEFINITIONS

AA	Abdominal Aorta
AAST	American Association for the Surgery of Trauma
Ab	Antibiotics or Antibody
Acidosis	Increased acidity in the blood
ACS	Acute Coronary Syndrome
ACS	American College of Surgeons
ACT	Activated Clotting Time
ADP	Adenosine diphosphate
AF	Atrial Fibrillation
ALP	Alkaline Phosphatase
AMI	Acute Myocardial Infarction
AO	Arbeitsgemeinschaft für Osteosynthesefragen
APC	Activated Protein C, anticoagulant, inactivates FVIIIa and FVa
aPTT	Activated Partial Thromboplastin Time, intrinsic pathway coagulation.
ARDS	Acute Respiratory Distress Syndrome
AT	Antithrombin
AT III	Antithrombin III inhibitor of thrombin, FIXa, Xa, XIa, Heparin target
ATC	Acute Traumatic Coagulopathy
ATLS	Advanced Trauma Life Support
ATP	Adenosine Triphosphate
AU	Australia
AVCOR	ArterioVenous Carbondioxide Removal
BE	Base Excess
BP	Blood Pressure
Bradycardia	Slow heart rate, Low pulse
BR2	Bradykinin Receptor 2

BT	Bleeding Time
C1INH	C1 esterase Inhibitor
C3	Complement factor 3
C5	Complement factor 5
CA	Canada
Cannula	A tube inserted in the body for removal or infusion of liquid, often blood.
Cannulation	The insertion of a cannula
Ca <sup>2+</sup>	Calcium Ion
CDH	Congenital Diafragmatic Hernia
CFT	Clot Formation Time. Time to fibrin polymerization
CH	Switzerland
Chemotaxis	The movement of an organism in response to a chemical stimulus
CNS	Central Nervous System
Coagulopathy	Dysfunctional coagulation ability
CO	Cardiac Output
CO <sub>2</sub>	Carbon Dioxide
Coagulopathy	Decreased coagulative/clotting ability INR<1.2, aPTT>35 s
COT	Committee on Trauma
COX	Cyklooxygenase, converts fatty acids to prostanoids
CPB	Cardiopulmonary Bypass
CPR	Cardiopulmonary Resuscitation
CRRT	Continous Renal Replacement Therapy
CT	Clotting Time
CT	Clotting Time. Initiation of clotting. Thrombin Formation
CT	Computed Tomography
CTI	Corn Trypsin Inhibitor
CVP	Central Venous Pressure
CVP <sub>inf</sub>	CVP in Inferior Vena Cava
CVP <sub>sup</sub>	CVP in Superior Vena Cava

DBP	Diastolic Blood Pressure
DCR	Damage Control Resuscitation
DCS	Damage Control Surgery
DIC	Disseminated Intravascular Coagulation
DO2	Systemic Oxygen Delivery
DTI	Direct Thrombin Inhibitor
DTSC	Definitive Trauma Surgical Care
DVT	Deep Vein Thrombosis
ECCOR	Extracorporeal Carbon Dioxide Removal
ECMO	Extracorporeal Life Support
ECMO	Extracorporeal Membrane Oxygenation
ECPR	Extracorporeal Cardiopulmonary Resuscitation
ECS	Extracorporeal Support
EDTA	Ethylenediaminetetraacetic acid
ELSO	Extracorporeal Life Support Organization
Endothelium	Inner layer of a blood vessel
ER	Emergency Room
Erythrocyte	Red Blood Cell
etCO2	End tidal carbon dioxide
ETP	Endogenous Thrombin Potential
EXTEM	Extrinsic Thromboelastometry
F	Coagulation Factor
FAST	Focused Abdominal Sonogram for Trauma
FDP	Fibrin Degradation Products
FEIBA	Factor Eight Inhibitor Bypassing Activity
FFP	Fresh Frozen Plasma
FI	Fibrinogen, Adhesive protein that forms the fibrin clot
Fibrin	Protein that stabilizes platelets in blood clots
Fibrinogen	Acute phase protein, cleaved by thrombin to fibrin

Fibrinolysis	Degradation of fibrin
FIBTEM	Fibrin Thromboelastometry
FII	Prothrombin
FIIa	Thrombin, Activated prothrombin, the main enzyme of coagulation
FIII	Tissue Factor, Thromboplastin, Initiator of extrinsic pathway
FiO2	Fractional inspired Oxygen Concentration
FIV	Calcium, Metal cation necessary for coagulation reactions
FIX	Christmas factor, enzyme for intrinsic activation of FX
FV	Labile factor, Cofactor for FX to activate prothrombin to thrombin
FVII	Proconvertin, TF and FVIIa initiates extrinsic pathway
FVIII	Antihemophilic factor, cofactor for intrinsic activation of fX
FX	Stuart-Prower factor, activates prothrombin, final common pathway
FXI	Plasma thromboplastin antecedent, intrinsic activator of factor IX
FXII	Hageman factor, initiates intrinsic pathway
FXIII	Fibrin stabilizing factor, transamidase that cross-links fibrin clot
GP	Glycoprotein
GSW	Gun Shot Wound
HA	Hemostatic Agent
Hb	Hemoglobin
HCT	Hematocrit
HD	Hemodialysis
HFOV	High Frequency Oscillatory Ventilation
Histamine	Increases the permeability in capillaries and proteins
HK	High Molecular Weight Kininogen, cofactor for activation of prekallikrein
HPF	High Power Field Image. Quantifies data in Electron Microscopy
HR	Heart Rate
HSPG	Heparan Sulfate Proteoglycan, endothelial cofactor to Antithrombin III
Hyperfibrinolysis	Increased fibrin degradation
Hyperlipidemia	High lipid levels

Hypocoagulable	Reduced coagulation capacity
Hypoperfusion	Reduced perfusion, blood flow
Hypovolemia	Low volume
Hypotension	Low blood pressure
Hypothermia	Low temperature
IAT SIC	International Association for Trauma Surgery and Intensive Care
ICU	Intensive Care Unit
Ig	Immunoglobulin
IJV	Internal Jugular Vein
IL-1/IL-6	Interleukine 1 and 6
In vitro	“In the glass”, tests with cells or biological molecules
In vivo	“Within the living”, tests on living organisms
INR	International Normalized Ratio, a Ratio of prothrombine time
INTEM	Intrinsic Thromboelastometry
IPPV	Intermittent Positive Pressure Ventilation
ISI	International Sensitivity Index
ISS	Injury Severity Score or International Society of Surgeons
IVC	Inferior Vena Cava
LA	Left Atrium
LAC	Lupus Anti Coagulant, Ig that binds to PL and increases clotting
Leukocyte	White blood cell
LMWH	Low Molecular Weight Heparin
LPK	Leukocyt Partikel Konzentration
LV	Left Ventricle
mAb	Monoclonal Ab. Made by identical cells cloned by unique parent cell
MAP	Mean Arterial Pressure or Mean Airway Pressure
MAS	Meconium Aspiration Syndrome
MASH	Mobile Army Surgical Hospital
MBT	Massive Blood Transfusion > 4 PRBC/1h or 50% of blood volume/3h

MCF	Maximum Clot Firmness. Clot stabilization by Fibrin, PLT and FXIII
MEDEVAC	Medical Evacuation, evacuation of a patient from the battlefield
ML	Maximum Lysis. Stability of the Clot or Fibrinolysis
mL	Milliliter
mm Hg	Millimeter Mercury (pressure unit)
MOF	Multi Organ Failure
Monocyte	Leukocyte in the immune system, can differentiate to a macrophage
MRI	Magnetic Resonance Imaging
NICU	Neonatal Intensive Care Unit
NIV	Non Invasive Ventilation
NO	Nitric Oxide, powerful vasodilator with halflife of seconds
Noradrenaline	Increases Heart rate and Blood pressure during stress
O <sub>2</sub>	Oxygen
OI	Oxygenation Index
OR	Operating Room
OT	Operating Theatre
PA	Pulmonary Artery
PAF	Platelet Activating Factor initiates platelet-aggr. and degranulation
PAI-1	Plasminogen Activator Inhibitor 1
Part.thrombopl.	Partial thromboplastin. Phospholipids without TF
PC	Protein C
PCC	Prothrombine Complex Concentrate. FII, FVII, FIX and FX
PCO <sub>2</sub>	Partial Pressure of Carbon Dioxide
PDGF	Platelet Derived Growth Factor
PE	Pulmonary Embolism
PEA	Pulseless Electric Activity
PEEP	Positive End Expiratory Pressure
PEG	PolyEthylene Glycol
PEO	PolyEthylene Oxide



Perfusion	The passage of a fluid through a vessel or an organ
PGE2	Prostaglandin E 2, Direct vasodilator
PGI2	Prostacyclin, Anticoagulant, Vasodilator and Platelet Act. Inhibitor
Phage Display	A technique to find Ab:s that fit certain proteins, e.g coag. factors
PHT	Pulmonary Hypertension, PA pressure > 25 mm Hg
PICU	Pediatric Intensive Care Unit
PK	Plasma Kallikrein
PL	Phospholipid
PLT	Platelet, Thrombocyte
PO2	Partial Pressure of Oxygen
PP	Pulse Pressure (SBP-DBP)
PPK	Plasma Prekallikrein, Fletcher factor, initiation of intrinsic pathway
Pport	Pressure in Portal Vein
PPP	Platelet Poor Plasma
PRBC	Packed Red Blood Cells
Prostacyclin	PGI2 Active in the resolution phase of inflammation
Prostaglandin	Mediator of inflammation and Anaphylaxis
Prostanoids	Prostacyklins, prostaglandins and thromboxanes
Protein C	Anticoag Act. to APC, downreg Thrombin, cleaves FVa and FVIIIa
Protein S	Cofactor for Protein C
PT	Pro-thrombin Time, measures the extrinsic pathway of coagulation
PVC	Poly Vinyl Chloride
Qport	Flow in portal vein
RA	Right Atrium
RCT	Randomized Clinical Trial
Recombinant	A product of genetic engineering
Ref.	Reference
ROTEM	Rotational Thromboelastometry
RPM	Revolutions Per Minute

RR	Respiratory Rate
RV	Right Ventricle
SBP	Systolic Blood Pressure
SBT	Skin Bleeding Time
SEM	Scanning Electrone Microscopy
SER	Serotonin
Serine	Aminoacid
Serine protease	Enzyme that cleave proteinbonds were serine is in the active site
Sic!	Sic erat scriptum. It is true!
SIRS	Systemic Inflammatory Response Syndrome
SO2	Arterial oxygen saturation
STRATEVAC	Strategic Evacuation, evacuation of patient to another country
SVC	Superior Vena Cava
SVO2	Mixed Venous Oxygen saturation
TA	Topical Agent
Tachycardia	High heart rate, Fast pulse
Tachypne	High respiratory rate
TAFI	Thrombin Activatable Fibrinolytic Inhibitor
TCR	Trans Capillary Refill
TEG	Thrombolestography
TEM	Thromboelastometry
TF	Tissue Factor
TFPI	Tissue Factor Pathway Inhibitor, Inhibits the TF/FVIIa complex
Thrombocyte	Platelet
Thrombokinase	Converts prothrombin toThrombin
Thrombomodulin	A cofactor for thrombin induced act. of protein C to anticoag. APC
Thromboplastin FIII,	Tissue Factor (TF)
TIC	Trauma Induced Coagulopathy
TNF	Tumour Necrosis Factor

tPA	tissue Plasminogen Activator
TPK	Trombocyt Partikel Konzentration, Platelet count
TVE	Total Vascular Exclusion
TXA2	Thromboxane A2, vasoconstrictor
UK	United Kingdom
UNFH	Unfractionated Heparin, "Heparin"
UO	Urinary Output
USA	United States of America
USG	UltraSonoGrapghy
VA	Venoarterial
VAD	Ventricular Assist Device
Vasopressor	Antihypotensive agent
VAV	Venoarteriovenous
Western Blot	Method to detect proteins in tissue samples, gel-electrophoresis + Ab:s
VO2	Systemic oxygen consumption
VTE	Venous Thromboembolism
VV	Venovenous
VVA	Venovenoartrial
vWD	von Willebrand Disease
vWF	von Willebrand Factor
WWI	World War I
WWII	World War II
ZA	South Africa (Republiek Zuid-Afrika)
Zymogen	Proenzyme, Inactive enzyme precursor



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“Factus est Dominus protector meus”

*In the early morning, on the 17<sup>th</sup> of June 1682, at Stockholms Castle, Queen Ulrika Eleonora gave birth to a Prince with a caul “segerhuva”. He was to become King Karl XII, responsible for even more blood shed on the soil of northern Europe...*



## **10. ARTICLES I-IV**