



PP2 Fibrinolysis and Fibrinogen

PP2-1

An integrated approach to effective Pathogen reduction for a plasma-derived Fibrinogen concentrate

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Objectives: The integrated approach to manufacture the fibrinogen concentrate Haemocomplettan P is assessed with regard to pathogen safety.

Design and Methods: Special care is taken to minimize the risk of transmitting blood-borne pathogens by (i) careful selection of plasma collection centres, donors, and donations (ii) testing the plasma pool for fractionation and (iii) ensuring adequate capacity of the production process to reduce a wide range of viruses as well as prions. The pathogen reduction capacity of the manufacturing process was studied employing the relevant blood borne viruses or specific and non-specific model viruses. In addition, the prion reduction capacity of the manufacturing process of Haemocomplettan P was investigated.

Results: The experimental data demonstrate for Haemocomplettan P a mean overall reduction of > 12.4 log₁₀ for HIV, > 12.7 log₁₀ for BVDV (model virus for HCV), > 10.7 log₁₀ for herpesviruses (unspecific model virus), > 7.7 log₁₀ for HAV and 6.0 log₁₀ for CPV (model virus for resistant non-enveloped viruses); furthermore, pasteurisation alone (heat treatment in aqueous stabilised solution at 60°C for 20 h) inactivated WNV (West Nile virus) and B19V (parvovirus B19) by > 8.3 log₁₀ and > 4.5 log₁₀, respectively. The prion reduction capacity of the manufacturing process was demonstrated to be 5.2 log₁₀ (microsomal fraction) and > 6.4 log₁₀ (purified PrP^{Sc}).

Conclusions: The measures taken are considered effective for enveloped viruses such as HIV, HBV and HCV and for the non-enveloped viruses HAV and parvovirus B19. In addition, the manufacturing process results in an effective prion reduction.

PP2-2

Combined thrombolysis with intravenous abciximab and intra-arterial alteplase yields high recanalization rate in patients with basilar artery occlusion

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Objectives: Recanalization of the basilar artery is crucial for improved functional outcome. We sought to determine the feasibility and efficacy of combined intravenous (IV) thrombolysis with the glycoprotein IIb/IIIa receptor inhibitor abciximab and intra-arterial (IA) thrombolysis with alteplase (rtPA).

Design and Methods: We prospectively studied patients with acute basilar artery occlusion on CT angiography (CTA) within 24 hours from symptom-onset. We treated patients with combined IV abciximab (0.25mg/kg bolus, followed by 0.125 µg/kg/min over 12 hours) followed by IA rtPA (up to 40mg or until recanalization). Primary outcome measure was complete or partial recanalization according to TIMI 2 to 3 flow grades on digital subtraction angiography (DSA). Secondary outcome measures were favourable functional outcome (modified Rankin Scale [mRS] score ≤3) and mortality at 90 days.

Results: We treated 20 patients: mean age 62±13 years, median baseline National Institutes of Health Stroke Scale (NIHSS) score 25.5, Glasgow Coma Scale (GCS) score 7, mean onset-to-treatment time 8.7±6.6 hours. We achieved partial or complete recanalization in 16/20 patients (80%). At 90 days, 3/20 patients (15%) had a favourable functional outcome and 11/20 patients (55%) were deceased. All 3 patients with a favourable outcome had complete (n=2) or partial (n=1) recanalization of the basilar artery.

Conclusions: Combined thrombolysis with IV abciximab and IA rtPA was feasible and resulted in a high recanalization rate. However, functional outcomes in our study were poor, potentially due to late initiation of treatment. Recanalization of the basilar artery was essential for a favourable functional outcome.

PP2-3

Comparison of plasmin generation and internal fibrinolysis in two groups of women suffering spontaneous abortions related or not to the antiphospholipid syndrome

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Antiphospholipid syndrome (SAP) can be the cause of fetal wastage due to the presence of unusual antibodies directed to proteins associated to anionic phospholipids. The aim of the present work was to compare the fibrinolysis process in a small group of women positive and negative for SAP.

Design and Method: Patients with SAP+ (n=12) were selected according to the revised Sapporo Criteria (2006), a commercial LA plasma was included. The SAP-patients (n=6) had spontaneous abortions of unknown etiology. The control group (n=12) was similar in age, fibrinogen concentration and body mass index to the patients groups, and was selected from the staff of our department. Fibrin polymerization and internal fibrinolysis was followed by turbidity. Plasmin generation was performed by chromogenic substrate S-2251.

Results: Characteristics of the SAP+ group: The SAP+ group had 1 patient positive for anti-β₂-GPI, 4 patients with anti-β₂-GPI and aCL, and 11 with aCL. Polymerization, Fibrinolysis and plasmin generation: The fibrin polymerization and internal fibrinolysis process was similar between the patients groups and controls. Only the commercial LA had a prolonged lag time (+ 25 sec), increased final turbidity (+ 0.11) and increased rate of clot dissolution (+ 292 sec) compared to control. Plasmin formation was significantly increased (p<0.05) in the SAP- group.

Conclusions: The number of subjects studied in the present work limits solid conclusions. However, the fibrin formation and clot dissolution of the patients studied in the present work was normal but different in LA.

PP2-4

Installation and implementation of a web-based data-acquisition system for evaluation of Fibrinogen-concentrate in the therapy of acquired perioperative coagulation disturbances

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Objectives: Severe traumatic or perioperative bleeding necessitates acute fluid therapy which has been shown to cause dilutional coagulopathy. The first compo-

ment to hereby be critically reduced is often fibrinogen. Despite that fibrinogen-concentrate has a valid allowance for the therapy of hereditary and acquired fibrinogen deficiency, its safety and efficiency in the therapy of bleeding-induced coagulopathy has never been evaluated in controlled clinical trials. In this patient collective, prospective intervention studies are limited due to the unpredictability of bleeding occurrences. Above that, an establishment of a placebo group is ethically highly problematic.

Methods: An improvement of the currently available data on the safety and efficiency of fibrinogen-concentrate during perioperative coagulation disturbances should be achieved by the construction of a web-based, retrospective case collection-system, in which completed therapies with fibrinogen-concentrate should be documented.

Results: For a short time, our case-collection system is available under www.fibrinogen-netzwerk.de. Data-implementation occurs password-protected after online registration and verification by the administrators. Only routinely acquired, anonymised data such as the cause and kinetic of the bleeding event, coagulation parameters before and after therapy, the kind of coagulation therapy, clinical effects of the therapy, adverse events as well as potentially occurring thrombo-embolic complications are recorded. The self-explaining and intuitively operating menu navigation accounts for a shortest possible expenditure of time.

Conclusions: By means of the web-based data-acquisition system, clinics of all specialities can participate. This should generate high case numbers in short time with relatively low logistical and financial efforts in order to derive statistically valid conclusions.

PP2-5

Validation of the Auto-Dimer® test on BCS®/BCS® XP Analyzers

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Background and Aims: AutoDimer® (Trinity Biotech, Ireland) is a rapid fully automated, latex-enhanced turbidimetric test for the quantitative determination of cross-linked fibrin degradation products (D-Dimer) in human plasma. Objective of this study was to validate its use on non Trinity Biotech analyzers, and to verify safety and efficacy of the assay in blood samples of patients with suspected deep venous thrombosis (DVT).

Methods: The validation procedure was performed according to NCCLS guidelines. Basic performance characteristics of the AutoDimer® was measured on two different analyzers (Siemens BCS® and BCS®XP), comparing results from clinical and healthy samples with those obtained using a comparable reagent (Siemens D-Dimer Plus).

Results: Within-run and total imprecision of AutoDimer® exhibited low imprecision (CV 0.7–7.5% and 1.9–8.2%, respectively) for both low and high levels of quality control. The correlation between AutoDimer® and D-Dimer Plus was good ($r > 0.89$). AutoDimer® levels in patients with DVT were significantly higher (BCS: 1642 µg/l; BCS-XP: 1684 µg/l) than those from the D-Dimer Plus (BCS: 526 µg/l; BCS-XP: 592 µg/l). Accuracy was good to excellent, with variation coefficients < 6%. Sensitivity was high (limit of quantitation < 50 µg/l). A high-dose hook-effect could be excluded up to a D-Dimer concentration to 100.000 µg/l. AutoDimer® was shown to be unsusceptible to interferences (haemoglobin, bilirubin, lipids, and heparin).

Conclusions: The AutoDimer® assay gives low imprecision, highly accuracy, sensitivity, and insusceptibility to interfering substances. Compared to the D-Dimer Plus, AutoDimer® showed a better separation between healthy and DVT patients by providing a significantly higher sensitivity.

PP2-6

Polymorphonuclear leucocyte elastase (ELP) and bacterial proteases degrade fibrinogen in severe bacterial infections.

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Objectives: Elastase and bacterial proteases play an important role in the course and prognosis of severe bacterial infections. We therefore measured the ELP-specific fibrinopeptide FpBb(30–43) in 25 patients and correlated the findings with other activation parameters and platelet count. We also tested the fibrinolytic activity of cell lysates of different bacterial strains, often found in septicemia.

Methods: FpBb(30–43) was determined by a radio immune assay according to Wallin and Saldeen (normal 400 pmol/l). ELP-a1Antitrypsin (AT) and Thrombin-Antithrombin (TAT) complexes were determined by ELISA techniques. The fibrinolytic activity of cell lysates of 6 different bacterial strains was measured with fibrin plates (Astrup).

Results: In 25 patients (group 1) with septicemia the mean initial value of FpBb was 3650 pmol/l and 1063 after recovery; the corresponding values in a group of 9 patients (group 2) with lethal course were 1937 and 2137, respectively. There was a good correlation with platelet count: 53 and 270 G/l (group 1) and 37 and 38 G/l (group 2), whereas TAT and ELP-a1AT complexes returned to normal in both groups in the course of disease. Bacterial strains exhibited different high fibrinolytic activities which only could be reduced by a2-macroglobulin.

Conclusion: FpBb and ELP-a1AT are highly elevated in the initial phase of severe bacterial infections as sign of in vivo proteolysis by ELP. FpBb stays pathologic in lethal outcome though ELP-a1AT has returned to normal. This shows that in these states elastase activity must still be present and cannot be inactivated by a1AT.

PP2-7

Local intraarterial thrombolytic therapy of an upper limb acute arterial thrombosis in a patient with severe, active Crohn colitis.

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Objectives: The prevalence of thrombosis is 6.2%, the average incidence of thromboembolic events (TE) are 3.6 times higher in patients with inflammatory bowel diseases (IBD), compared to normal population. The TE is squarely associated with the IBD activity. The application of anticoagulant and thrombolytic therapy in severe IBD is an unsolved issue.

Design and Methods: A 46-year-old male patient is presented who had Crohn colitis. Endoscopy showed extended, severe inflammation of the colon with deep ulcers and signs of chronic activity. Besides steroids he took sulfasalazine, mesalazine then azathioprine. He was treated with high dose prednisone due to severe activity of IBD in December 2007. In March 2008, his right hand suddenly became cold and painful. Angiography proved acute occlusion of the brachial and radial artery. Vascular surgery intervention was not applicable. Despite of the severe endoscopic findings, frequent bloody stools and significant anemia, local intraarterial thrombolytic treatment was initiated considering the severity of the occlusion. For intervention rt-PA was applied for 9 hours, the therapy was continued with LMWH.

Results: The control angiography proved improvement, the radial artery pulse appeared. No bleeding complication was observed. The source of the embolus was undetectable.

Conclusions: Thrombolytic therapy in severe, active IBD is an unsettled question. If the indication is strong, thrombolysis is not contraindicated in TE associated with active IBD. In IBD TE risk should be estimated and thromboprophylaxis considered. As microvascular thrombosis plays a role in the pathomechanism of IBD, adequate anticoagulant prophylaxis may promote the remission of the disease.

PP2-8

Reduced fibrinolytic activity in women after delivery in Benin City, Nigeria.

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Fibrinolytic activity (using ELT) and plasma fibrinogen concentration in 50 mothers just after delivery were assessed (giving 50 mother's and 50 cord samples). Also 50 healthy women without delivery were used as controls. All these were to ascertain if there is any change in fibrinolytic activity in women after delivery.

Methodology: Fifty women (22 – 35 years) with normal delivery were used for this study (150 samples). Blood samples were collected and investigations were performed before noon. ELT (fibrinolytic activity) was carried out using the method of Cecil Hougie (1986) and plasma fibrinogen concentration (PFC) was estimated using Ingram clot weight method (1961). The t-distribution test was used to test for level of significance between mean of both test and controls, $P < 0.05$ was significant.

Results: The results show significantly lower fibrinolytic activity in mothers and significantly higher fibrinogen concentration than controls ($P < 0.05$). Maternal PFC and ELT values were also significantly higher than cord samples.

Conclusion: There is reduced fibrinolytic activity and increased fibrinogen concentration in mothers immediately after delivery. This may be a protective coagulative mechanism against excessive post partum haemorrhage.