Endothelial- and Platelet-derived Microparticles are generated during Liver Resection in Humans

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Abstract

Background: Cell-derived plasma microparticles (<1.5 µm) originating from various cell types have the potential to regulate thrombogenesis and inflammatory responses. The aim of this study was to test the hypothesis that microparticles generated during hepatic surgery co-regulate post-operative pro-coagulant and pro-inflammatory events.

Methods: In 30 patients undergoing liver resection, plasma microparticles were isolated, quantitated and characterised as endothelial (CD31+, CD41-), platelet (CD41+) or leukocyte (CD11b+) origin by flow cytometry and their pro-coagulant and pro-inflammatory activity was measured by immunoassays.

Results: During liver resection the total numbers of microparticles increased with significantly more Annexin V-positive, endothelial and platelet-derived microparticles following extended hepatectomy compared to standard and minor liver resections. After liver resection, microparticle tissue factor and pro-coagulant activity increased along with overall coagulation as assessed by thrombelastography. Levels of leukocyte-derived microparticles specifically increased in patients with systemic inflammation as assessed by C-reactive protein but are independent of the extent of liver resection.

Conclusions*:* Endothelial and platelet derived microparticles are specifically elevated during liver resection, accompanied by increased pro-coagulant activity. Leukocyte-derived microparticles are a potential marker for systemic inflammation. Plasma microparticles may represent a specific response to surgical stress and may be an important mechanism of postoperative coagulation and inflammation.

Key words: Microparticles, liver resection, platelets, endothelium, patients

Introduction

Initially described as “platelet dust” in 1967[1], platelet-derived microparticles (MP) and other circulating cell-derived MP have been recognized as important mediators of inflammation and coagulation. These MP are of potential relevance for extracellular cell signalling and have been shown to be important in different disease states[2].

Vascular and abdominal surgery provokes a local and systemic pro-inflammatory and pro-coagulant stimulus[3], catalyzing an acute phase reaction for several days post-intervention. Post-operative hypercoagulability may manifest itself as venous or arterial thrombotic events and is associated with significant morbidity, mortality and costs[4]. In addition to platelets, a variety of MP play a decisive role in coagulation[5-6]. In particular platelet-derived, endothelium-derived and leukocyte-derived MP contribute to local and systemic coagulation[7] by expressing a variety of surface markers such as phosphatidylserine, CD61, P-selectin and factor X, interact with glycoprotein ligand-1 to localize tissue factor, β2-integrins or ICAM-1[8-9]. In vivo, MP influence endothelial dysfunction in patients with myocardial infarction and metabolic syndrome[10-11], whilst recent data suggest a clear association of circulating MP with cardio-metabolic risk factors, in particular dyslipidemia, highlighting effects on endothelial integrity[12]. Increasing evidence suggests MP may be relevant in many settings of inflammation and organ injury and possibly represent a biomarker for various diseases, such as septic shock-induced disseminated intravascular coagulation[13] and atherosclerosis[14]. Not only the number of MP is changed in the setting of disease, but also their function, as a recent study has shown that MP from patients with Crohn’s disease significantly alter endothelial and vascular function[15]. Recent literature reveals a significant impact of the release of microparticles and alterations in coagulation in trauma patients and in patients undergoing cardiac or vascular surgery[16-17].

The liver is the main organ regulating systemic inflammation and coagulation, however data regarding generation of MP in the context of liver disease is scarce. It has been reported that MP released from liver cells potentially modulate cirrhosis and cirrhotic vasculopathy[18-20]. In the context of liver transplantation, increased hepatocellular damage, platelet activation, as well as augmented levels of platelet MP were observed in the post-operative period in the patient group with elevated markers of ischemia and reperfusion injury[21] and correlate with acute liver failure[22]. Furthermore, a recent study in a murine model of acute hepatic ischemia and reperfusion injury suggests specific roles for microparticle subsets in modulating the response to reperfusion injury[23]. Currently no data exist on how extensive liver surgery other than transplantation, with particular regard to extent of surgical intervention and hepatic inflow occlusion, affect MP generation and distribution.

The aim of the current study therefore was to test the hypothesis that MP generated during hepatic surgery co-regulate post-operative pro-coagulant and pro-inflammatory events.

Materials and methods

Study design and patient collection

According to the guidelines for good clinical practice, the study protocol was approved by the institutional review board and cantonal ethics committee of Bern, Switzerland. All patients gave written informed consent prior to study inclusion. he study was conducted according to the principles expressed in the Declaration of Helsinki. Eligible patients were at least 18 years of age and underwent planned, non-emergency liver and pancreatic surgery. Thirty patients undergoing liver surgery and thirteen patients undergoing pancreatic surgery (as controls) were enrolled in the study. We hypothesised that patients undergoing pancreatic surgery undergo significant changes in the coagulation system compared to patients undergoing liver surgery. The liver surgery population comprised 13 women and 17 men, with an average age of 58 years (range 30-81). Indications for an operative intervention included surgery for metastasis (n=11, 37%), hepatocellular carcinoma (n=6, 20%), cholangiocellular carcinoma (n=4, 13%) and surgery for benign liver disease (e.g. hepatic adenoma, n=9, 30%). Segmentectomy was performed in five patients (17%), left hemihepatectomy in three patients (10%), central resection in four patients (13%), right hemihepatectomy in ten patients (33%) and extended hemihepatectomy in eight patients. All patients received prophylactic doses of subcutaneous weight-adapted low molecular weight heparin peri-operatively. No other anticoagulants were specifically used in the immediate pre- or post-operative period.

Collection of blood samples for microparticle isolation

Venous blood was collected from a central line at various time points into ethylenediaminetetraacetic acid (EDTA) anti-coagulated sterile syringes. Cells were

removed by centrifugation (15 minutes at 1500 g at 20 °C). Platelet poor plasma (PPP) was generated by renewed centrifugation (1 minute at 20000 g), samples aliquoted and stored at -80 °C until use.

Isolation of microparticles

All PPP samples of one patient were tested in the same experiment to avoid day-to-day variations of the flow cytometer. PPP samples were slowly defrosted for 45 minutes in ice water. After careful mixing, 250 µl of PPP was transferred into a clean Eppendorf tube. MP were concentrated in two spin-down steps (30 minutes at 20000g at 20 °C each) and finally re-suspended in 75 µl citrate/phosphate-buffered saline (PBS).

Reagents

All reagents and antibodies were diluted in a calcium chloride/PBS solution ($CaCl_2$ 2.5 mmol/L, pH 7.4, 0.2 µm filtered). The optimal antibody concentration was determined by titration. The following reagents and antibodies were used: Annexin V-Cy5-APC (Allophycocyanin, MBL international Corporation, detection of phosphatidylserine, 1:300), CD31-FITC (Fluorescein isothiocyanate, eBioscience, monoclonal mouse anti-human, 1:1000), CD41-PE (Phycoerythrin, Dako, monoclonal mouse anti-human, 1:200) and CD11b-PE (BD Biosciences, USA, monoclonal mouse anti-human, 1:100). Isotype controls used were IgG1-PE, IgG2a-PE and IgG1-FITC (all from DAKO). For the Annexin V-APC negative control, Annexin V-APC was diluted 1:300 in citrate/PBS solution. Endothelial MP were defined as CD31+ (CD41-), platelet as CD41+ (showing weak CD31+) and leukocyte MP as CD11b+.

Flow cytometric analysis of microparticles

All tests were performed with triple staining in duplicates. MP-suspensions were incubated with antibodies for 30 minutes at room temperature in the dark. The reaction was stopped by adding 900 µl citrate/PBS solution. Samples were analysed by BD LSR II flow cytometer and FlowJo Computer Software (Version 8.8.2 for Mac). Prior to measurement, the cytometer was subjected to a 30 minute wash process to minimise background noise. Forward and sideward scatter were set at logarithmic gain (Figure 1A and B). The measurement time for each tube was set at 90 seconds. MP were identified by size <1.5 µm (size calibration using TetraSpeck microspheres 1.5 µm, Invitrogen, Figure 1A and B) and primarily gated for Annexin V-APC positive events. This population, identified as MP were stained for cell membrane markers to determine cellular origin (Figure 1C). Numbers were corrected for haemodilution.

Multiplex analysis of microparticle-rich plasma

A Bio-Plex human cytokine assay for simultaneous quantitation of nine cytokines (IL-1ß, IL-6, IL-8, IL-10, IL-12, IL-13, IFN-γ and TNF-α) was performed in MP-rich plasma samples according to the manufacturer's instructions (Bio-Rad Laboratories, Hercules, CA). The tested parameters were measured by sandwich immunoassay using the Luminex fluorescent bead technology. Data analysis was done with Bio-Plex Manager version 4.0 software with five-parametric curve fitting (Bio-Rad Laboratories).

Analysis of MP pro-coagulant activity and MP tissue factor activity

To assess MP pro-coagulant activity and MP tissue factor activity in plasma, the commercial Zymuphen MP-activity immunoassay kits (Hyphen BioMed, France) were used.

For pro-coagulant activity, in brief, diluted platelet poor plasma samples were supplemented with calcium, Factor Xa and thrombin inhibitors and were introduced into microplate wells coated with streptavidin and biotinylated Annexin V. After an incubation and a washing step, the Factor Xa-Va mixture containing calcium was added, followed by addition of purified prothrombin. When present in the tested sample, on the surface of MP bind to Annexin V, subsequently allowing to Factor Xa-Va, in presence of calcium, to activate prothrombin to thrombin. The concentration of phospholipids represents the limiting factor. The phospholipid concentration and the amount of thrombin generation, which is measured via its specific activity on the thrombin substrate, directly correlate. Following stopping of the reaction with 2 % citric acid, the absorbance is measured at 405 nm.

To assess MP exposing surface-bound tissue factor platelet poor plasma samples were added to microplate wells coated with a murine monoclonal antibody specific for the extracellular domain of human tissue factor. Tissue factor bearing MP present in the sample bound to the immobilized antibody following overnight incubation. After washing, factor VIIa and factor X were added. The complex of tissue factor together with FVIIa leads to activation of FX to FXa on the surface of anionic phospholipids on the MP surface. Surface tissue factor on the MP are the rate-limiting step. A yellow chromogen is produced following the addition of a specific substrate for FXa and the absorbance, which is directly proportional to amount of MP tissue factor, measured at 405nm.

Soluble complement C3a and C5b-9 ELISA in platelet poor plasma samples

Soluble C3a and Cb5-9 levels were measured in PPP samples according to the manufacturer's guidelines (both Quidel, San Diego, CA, USA). In brief, samples and controls were incubated in a specific microtiter plate coated with a murine monoclonal

antibody specific for a neo-epitope on human C3a. Following washing, a horseradish peroxidase-conjugated polyclonal antibody to the C3a region of C3 and a chromogenic substrate were added and colour intensity measured spectrophotometrically at 450nm. For measurement of C5b-9, a similar procedure was followed, using a murine monoclonal antibody that binds specifically to the C9 ring of sC5b-9, followed by an HRP-conjugated polyclonal anti-C5b-9 antibody and chromogen and colour intensity was measured at 450nm.

Thrombelastography (TEG) Analysis

For TEG analysis, 1 ml of patient blood was added to a tube containing 1 % celite. The solution was mixed by inversion and 360 µl were transferred into a disposable cup and inserted into a computer-controlled TEG® 5000 Thrombelastograph® Hemostasis Analyzer (Haemonetics Corporation, Braintree, MA, US). The following TEG variables were measured for each sample: reaction time (r-time; normal range, 5.5-7.5 min), clot formation time (k-time; normal range, 1.5-3.5 min), angle (normal range, 54-74°), and maximum amplitude (MA; normal range, 55-68 mm). All normal ranges refer to celite-activated whole blood samples. The time needed to form a stable clot (TMA, time to maximal amplitude), the G-parameter as a measure of clot firmness and E-parameter as a measure of the elasticity constant were measured and expressed as dyn/cm^2. Clot lysis parameters including A30 and A60 (point measurements that look at TEG tracing amplitude; lysis at 30 and 60 minutes after maximum amplitude) and Ly60 (percent lysis 60 minutes after maximum amplitude) were also evaluated. A TEG coagulation index (CI) describing the subject's overall coagulation was derived from the r-time, k-time, MA, and angle. Positive values outside this range indicate that the sample is hypercoagulable; negative values that the sample is hypocoagulable.

Statistical Analysis

Concentration and type of MP were related the extent of liver resection over time. The impact of hepatic inflow occlusion was assessed in a post hoc analysis. A p-value of <0.05 was considered as the level of significance. Data were compared by use of one-way-analysis of variance (ANOVA) or, where appropriate, by use of two-way repeated-measures ANOVA, with correction for multiple testing. Data collection, data management, and data analysis were performed with the statistical package SPSS Version 15 (Chicago, IL). As this was primarily an observational study, no prior formal power analysis was performed. Instead, group numbers were chosen according to experience from previous work to take into account the expected range of inter-individual variation of MP numbers.

Results

Determination of microparticle (MP) numbers

MP derived from platelet poor plasma were stained with Annexin V and cell surface markers and analysed by flow cytometry (see materials and methods and representative dot blot in Figure 1).

Absolute MP numbers, defined as an Annexin V positive population in a log-log setting by flow cytometry increased both in the minor and standard resection population (n=22) as well as in the patients with extended liver resection (n=8) during the operation, decreasing by post-operative days one and three (Figure 2 A). As compared to patients undergoing minor and standard hepatic resection, the increase in total Annexin V positive MP numbers was significant in patients undergoing extended liver resection and did not recover down to baseline values up to day three (Figure 2 A). Endothelial cell derived CD31+ but not leukocyte derived MP numbers were significantly elevated in patients undergoing extended liver resection compared to patients undergoing minor and standard resection (Figure 2 B, C). Patients undergoing liver resection frequently undergo temporary inflow occlusion in order to control haemorrhage. In 10 patients that underwent inflow occlusion the fraction of platelet derived CD41+ MP was selectively elevated to 72.3±11% compared to 56.7±17% at baseline (Figure 2 D). There is no significant association of pre- or intraoperative microparticles with surgical site infection. Baseline values reveal a significant association between the frequency of endothelial derived CD31 positive microparticles and the presence of malignant versus benign disease (69% (SD 16%) vs. 58% (CD3)).

Plasma/microparticle cytokines are increased in the immediate post-operative period

MP containing platelet poor plasma from patients were analysed for cytokine levels (Figure 3). Unlike systemic cytokine levels these are likely in part MP-associated. Of the parameters tested, levels of IL-1ß, IL-6, IL-10 changed significantly during the course of the operation and in the immediate post-operative period. As compared to baseline values, levels of IL-1ß significantly increased at the immediate post-resection time point ($p=0.0001$) and remained significantly elevated as compared to baseline ($p=0.042$). IL-6 values continually increased from the immediate post-resection time point up to post-operative day one ($p=0.003$ and $p=0.006$ as compared to baseline), decreasing to non-significant levels thereafter (not shown). IL-10 values particularly increased significantly in the immediate post-operative phase ($p=0.009$), returning close to baseline thereafter, however, with a few outliers observed. The other cytokines measured (IL-8, IL-12, IL-13, IFN-γ or TNF-α) did not show significant changes in the course of the operation or early post-operative period as compared to baseline (values not shown).

C-reactive protein levels correlate with microparticle numbers

Patients were stratified according to CRP levels arbitrarily below or above 100 mg/l. CRP levels at postoperative day 2 inversely correlated with numbers of circulating CD31+ (CD41-) MP ($p=0.006$) (Figure 4A). Conversely, CRP directly correlated with leukocyte-derived MP ($p=0.039$) (Figure 4B). Although to a lesser extent, platelet-derived, CD41-positive MP were detected in circulation in the CRP “high” as compared to the “low” group, although this was not statistically significant (Figure 4C).

Microparticle tissue factor activity and total microparticle pro-coagulant activity are increased during surgery whilst complement C3a initially decreases in major liver surgery

In the whole study population, MP tissue factor activity was significantly increased during the operative period as compared to baseline (p=0.017 at 2 hours, p=0.004 at 4 hours and p=0.006 at 6 hours, Figure 5A). Interestingly, MP tissue factor activity dropped during the first day post-surgery and rapidly thereafter, with values in the post-operative period no longer significantly different as compared to baseline. Activation of complement, measured as soluble C5b-9 in plasma samples by ELISA, was highly variable but not significantly different between the time points and particularly as compared to baseline (p>0.05, Figure 5B). Another soluble complement activation product, the anaphylatoxin C3a, did also not show any significant changes throughout the intra- and immediate post-operative period, when grouping all patients together (p>0.05, Figure 5C). However, if the patients were divided into minor and standard versus major liver resection, the patients in the major liver resection group revealed decreased levels of circulating C3a within the first few hours of the operation (p=0.015 at 4 hours and p=0.012 at 6 hours, Figure 5D). Overall, MP total pro-coagulant activity in all patients increased during surgery and up to day one post-operatively as compared to baseline (Figure 5E), recovering thereafter.

Overall coagulation parallels elevation of pro-coagulant MP activity

In order to compare MP pro-coagulant activity with overall coagulation, a TEG analysis was performed pre-operatively, during the operation and at day one post-operatively. The TEG coagulation index, which describes overall coagulation, increased during the operation as compared to baseline (p=0.003, Figure 6 A), with values dropping in the immediate post-operative period and at post-operative day one to return to baseline,

paralleling the increase in pro-coagulant MP activity already measured by immunoactivity assay.

In order to assess the influence of intraoperative manipulation of organs in the upper abdominal tract *per se* on TEG values, pancreatic resections were used as controls. TEG readings increased from a normal, negative value at baseline to a positive value at two hours after incision, indicating a slight trend towards hypercoagulability in this early intra-operative time point (Figure 6 B). Coagulation index was significantly different after hepatic resection compared to pancreatic resection. Significant differences were found after 2 hours, 4 hours and the elevation at 2h and decrease at 4 hours post incision was higher post hepatic (Figure 6 B). Ly60, indicating clot lysis 60 minutes after maximum amplitude (which represents ultimate strength of the clot and is a measure of platelet function) showed a bimodal distribution and was only elevated after pancreatic resection (Figure 6 C).

Standard laboratory tests

Laboratory values from patients undergoing standard and extended hepatectomy were measured at various time points. White and red blood cell concentration such as leukocyte count, haemoglobin concentration and thrombocyte count did not change significantly over time. Similarly measures of liver injury such as bilirubin, alanine amino transferase and coagulation assessed by prothrombin time did not change significantly over time (data not shown).

Discussion

Aim of the current study was to evaluate the impact of liver resection on the source and activity of circulating microparticles (MP). The main findings of the study reveal that MP are elevated during liver resection. In particular, absolute MP numbers and more specifically endothelial- and platelet-derived MP numbers were significantly elevated in extended as compared to standard and minor liver resections. These data suggest that overall MP count may appear to correlate with the extent of hepatic tissue resection. Furthermore, this study reveals that the subset of platelet-derived MP is specifically elevated after hepatic ischemia due to inflow occlusion. This is in line with previous data where, in the situation of liver transplantation, an increase in levels of platelet MP were observed post-operatively in the patient group with elevated markers of ischemia and reperfusion injury[21]. A large part of this increase, particularly in platelet-derived MP, is most likely due to increased shedding from activated platelets[22-24]. To which extent a reduced clearance may have contributed to this increase is not clear and remains to be investigated. Furthermore, our findings corroborate results from a very recent study in a murine model of hepatic ischemia and reperfusion injury, which revealed an elevation of circulating MP – in particular platelet and neutrophil MP[23]. Not only this current work, but also very recent studies currently raise the question whether the observed alterations in MP numbers in hepatic disease directly reflect the pathophysiological insult to the liver or compensatory responses, in acute (such as in this study) or also chronic liver injury[25]. Interestingly we found a significant association with the frequency of endothelial-derived microparticles and the presence of malignant versus benign disease (69% (SD 16%) vs. 58% (CD3)). These results support the hypothesis that microparticles may mediate angiogenic factors as shown in patients undergoing surgery for biliopancreatic malignancies[26], but requires further investigations.

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Pro-inflammatory profile post-liver surgery

Operative interventions represent a major pro-inflammatory and pro-coagulant stimulus. This is confirmed in the current study, revealing an augmented cytokine response in the early post-operative phase. Time point and elevation of the cytokine response corresponds in part to the elevated number (as detected by flow cytometry) as well as increased total pro-coagulant activity (as detected by immunoactivity) of the MP population. These data suggest that also in man, as previously shown in mice and in vitro [2, 27-28], MP may boast a role as carriers for cytokines, delivering pro- as well as potentially anti-inflammatory signals to target sites.

Thrombogenicity in the post-operative period

In this study we describe a transient state of hypercoagulability in the early postoperative phase of major uneventful abdominal surgery that was detected by MP coagulation index and thrombelastography but was not obvious in standard coagulation assays such as prothrombin time as assessed by INR. This hypercoagulability appears to be predominantly caused by a relevant elevation of platelet activity, which is in line with the finding that in addition to platelets, platelet-derived MP also play a decisive role in coagulation[29, 6]. Post-operative hypercoagulability may therefore more accurately be reflected by platelet activity and activation products i.e. platelet-derived MP than more commonly used coagulation assays. Whether this translates to and correlates with an increased risk for peri-operative thrombo-embolic events was presently not specifically addressed. However, up until release from hospital none of the patients presented with clinically manifest thrombo-embolic events.

In the study population total MP tissue factor activity, as measured by immunoactivity

assay, was increased in the immediate post-operative period. Interestingly, not only platelet-derived, but also leukocyte-derived MP importantly contribute to coagulation[7] in vivo by binding to activated platelets to localize tissue factor to and induce fibrin generation at the site of injury[9]. In the current study, leukocyte-derived MP numbers were also increased in the early post-operative phase, possibly indicating a role for leukocyte-MP in regulation of early post-operative coagulation events. Equally endothelial-derived MP interact with monocytic cells in vitro to stimulate tissue factor-mediated pro-coagulant activity, in part dependent on the interaction of intercellular adhesion molecule-1 on the MP and β2-integrins[26].

Soluble complement activation products C3a and C5b-9 were measured by ELISA in platelet poor plasma samples. Although differences were noted in plasma levels of C5b-9, these were not significant between groups (standard vs. extended liver resection) potentially because of the surgically induced reduction of total liver parenchyma, which typically synthesizes complement. The drop in leukocyte-derived MP may be explained by sequestered ectosomes (MP) derived from human polymorphonuclear neutrophils that activate and bind complement, possibly leading to clearance from the circulation much like the removal of circulating immune complexes[30].

Study limitations

One limitation of the study is the non-homogenous patient population undergoing liver surgery ranging from benign to malignant diseases. Previous studies have shown alterations in MP tissue factor activity samples in cancer patients[31-32]. The differences in MP pro-inflammatory and pro-coagulant activity in this study were not dependent on the initial condition with which the patient presented. The extent of the operation and

whether or not the intervention necessitated a phase of hepatic ischemia and reperfusion more importantly impacted on MP numbers and distribution. A lack of association of MP number with a defined clinical outcome parameter is another limitation. Nevertheless, the incidence of post-operative complications such as surgical site infections, biliary fistula or post-operative hepatic insufficiency is well below 10% and consequently patient number would need to be increased at least ten fold in order to determine good correlations. The current study does however show an association between MP numbers and CRP – a marker typically accepted as a good predictor of post-operative complications and outcome[33]. Because the field is still novel, analysis of MP, including the method of detection, determination of size and accurate gating in flow cytometry as well as quantification are currently not yet standardized[34-35]. And whilst flow cytometry currently largely remains the standard of MP analysis, the method in itself may inherently miss a possibly relevant number and population of MP not amenable to flow cytometric analysis (due to too small size). No significant association of microparticle count with surgical site infection was observed. However, this study was neither powered nor designed to address this question.

In summary MP are significantly elevated and ratios of platelet-, endothelial and leukocyte-derived MP are altered during major liver resection. These released and measurable circulating MP seem to represent a specific response to surgical stress and potentially exhibit specific thrombogenic and pro-inflammatory properties.

Disclosures

The authors declare they have no potential conflict of interest and no competing financial interests.

Author contributions

AV, DC and GB were in charge of patient care / operative interventions. YB, GMI, RR and GB designed and performed the experimental / laboratory work. YB, GMI and GB analysed the data and wrote the manuscript. All authors approved the submitted final version of the manuscript.

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Legends to figures

Figure 1

Flow cytometric analysis of circulating microparticles (MP) from platelet poor plasma.

Microspheres of 1.5 µM (black arrow) were used as size references to gate the MP population (defined as <1.5 µM) and regulate acquisition (A). Events falling in the MP gate (B) were gated for positivity for Annexin V. This population, identified as MP, was stained for cell membrane markers to determine cellular origin (C). Dot blots depict representative examples of actual blots used for subsequent analyses.

Figure 2

Relative number of total microparticles as measured by flow cytometry.

MP were assessed in patients undergoing liver resection for different conditions. Total number of circulating MP were elevated in patients undergoing extended liver resection compared to standard resection (A). Endothelial cell derived MP but not leukocyte derived MP were significantly elevated after extended **(n=8)** versus standard/minor **(n=22)** liver resection (B, C). Interestingly, platelet derived CD41-positive MP were significantly elevated at postoperative day one in patients in which temporary inflow occlusion compared to patients without inflow occlusion (D).

Figure 3

Measurement of cytokines in patient plasma during the peri-operative period.

Cytokines with pro-inflammatory properties (IL-1ß, IL-6) or anti-inflammatory properties (IL-10) were measured by ELISA from the patients' plasma (platelet poor plasma, MP-containing) before operation (baseline), after hepatic resection and at postoperative

day one. The levels of IL1ß were significantly elevated post resection (p=0.0001) and at postoperative day one (p=0.042) compared to baseline (A). Levels of IL-6 were significantly elevated post resection (p=0.003) and at postoperative day one (p=0.006) compared to baseline (B). Levels of IL-10 were only significantly elevated post resection (p=0.009) but not at postoperative day one compared to baseline (C)

Figure 4

Percentages of the individual microparticle subpopulations as measured by flow cytometry.

Representation of CD31-positive (A), CD11b-positive (B) and CD41-positive (C) microparticle (MP) populations, were compared according to C-reactive protein (CRP) values above or below 100mg/l. Significant inverse correlation of numbers of circulating CD31-positive MP with CRP (p=0.006). Significant direct correlation of leukocyte-derived MP with CRP (p=0.039). No significant correlation of platelet-derived, CD41-positive MP with CRP.

Figure 5

Measurement of microparticle-associated tissue factor, plasma levels of complement activation products and microparticle pro-coagulant activity.

Activation of tissue factor (A) and complement, measured by plasma levels of C5b-9 (B) and complement C3a in all patients (C) and in patients after extended liver resection (D) as well as microparticle (MP) pro-coagulant activity (E) were measured by immunoactivity rsp. enzyme-linked immunosorbent assay (ELISA). Significant increase of tissue factor activation was observed during the operation but not at later time points. Decrease of complement C3a activation was observed at 4 and 6 hours but not at the

other time points. Significant increase in MP pro-coagulant activity was noted during surgery and up to day one post-operatively as compared to baseline (p=0.005, E).

Figure 6

Thrombelastography measurements of patient blood samples.

Thrombelastography (TEG) was performed in order to correlate microparticle (MP) pro-coagulant activity with overall coagulation parameters and to compare between hepatic and pancreatic operations. The TEG coagulation index represents a composite score of various parameters assessed during TEG. Significant increase in coagulation index during the operative procedure was observed as compared to baseline (p=0.003), with values dropping in the immediate post-operative period and at post-operative day 1 (p=0.040, A). In order to compare for the intra-operative manipulation of abdominal organs, pancreatic resections were used as controls. Coagulation index was significantly more variable after hepatic resection as compared to pancreatic resection at early time points (B). Ly60, a marker for fibrinolysis, revealed significant responses in patients undergoing pancreatic resection but not following liver resection (C).