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## Factors associated with outcome of liver surgery and hepatocellular carcinoma

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# CHAPTER 10

## GENERAL DISCUSSION AND PERSPECTIVES

Chronic liver disease resulting in fibrosis and cirrhosis is associated with morbidity and mortality, irrespective of the etiology.<sup>1,2</sup> Although major progress has been made in the medical management of patients with liver diseases, liver surgery remains the only curative treatment strategy for many conditions. Yet, factors such as perioperative bleeding, and the capacity of remnant liver to regenerate may affect the outcome following the liver surgery.<sup>3-5</sup> In this thesis we conducted clinical and pre-clinical studies that aimed to gain better understanding of factors influencing the outcome of liver surgery. In addition, we evaluate if the development of HCC in patients with cirrhosis is associated with activation of primary hemostasis and whether or not the bioactive molecules within the platelets are altered in cirrhotic patients with hepatocellular carcinoma (HCC). In addition, we studied the paradoxical relation of gamma glutamyl-transferase (GGT) with outcome in liver transplantation patients and in patients admitted to the intensive care unit.

In chapter two, we discussed the surgical, anesthetic, and pharmacological strategies to reduce blood loss in patients undergoing a major liver surgery. Bleeding in liver surgery cannot be completely avoided because of the dual and large blood supply to the liver and because of retrograde bleeding from the sinusoids and hepatic veins during the parenchymal transection. Major blood loss requiring transfusion of blood or blood products is an independent risk factor for postoperative morbidity and mortality.<sup>4</sup> Generally, refinements in surgical techniques, anesthesiologic care, and better understanding of coagulation in patients with underlying liver disease have contributed to minimizing blood loss in liver surgery.

The introduction of maintaining a low central venous pressure (CVP < 5mm Hg) during the parenchymal transection, has been one of the key contributors of anesthesiologic techniques in the reduction of bleeding complication in liver surgery. A CVP < 5 mm Hg has shown to result in a four-fold reduction in bleeding and transfusion requirements compared to an anesthesiological management resulting in higher CVPs<sup>6</sup>. This mechanism is based on the pressure gradient between the CVP and liver sinusoids. The pressure within the liver sinusoids is related to the pressure in the hepatic veins, which subsequently, is directly related to CVP. The pressure within the sinusoids drops as consequence of clamping the inflow of blood to the liver through the hepatic artery and portal vein during parenchyma transection. A major disadvantage of lower CVP is the increased risk of complications such as air embolism, systemic tissue hypoperfusion, and renal insufficiency. However, most of these complications are transient and not (always) clinically relevant.<sup>7,8</sup>

Furthermore, better understanding of hemostasis especially in patients with cirrhosis has contributed significantly to a reduction of bleeding complications in liver surgery.<sup>9-13</sup> Many patients with advanced cirrhosis may have abnormal coagulation tests including an increased bleeding time, APTT or PT/INR. Historically, it was believed that abnormal coagulation assays are associated with an increased risk of bleeding complications. Hence, many centers

attempted to decrease blood loss and transfusion requirements by correcting the coagulation assays using whole blood or blood products.<sup>14</sup> Frequently, patients were fluid overloaded during the operation. Yet, no reduction in blood loss or transfusion requirements was achieved.<sup>15</sup> Paradoxically, the correction of coagulation assays resulted in significantly increased bleeding and transfusion requirements.<sup>16</sup> A common misconception has been the assumption that cirrhotic patients have a hemostasis-related bleeding tendency. This was based on the clinical observations that spontaneous bleeding occurs frequently in patients with cirrhosis, liver transplant recipients require considerable transfusion of blood products, patients have thrombocytopenia with functional platelet defects, and that conventional coagulation tests such as PT and APTT are frequently abnormal. It was believed that the increased risk of bleeding is resulted from an inherent hemostatic defect evidenced by both abnormalities in coagulation tests and clinical bleeding. However, bleeding complications are not only limited to cirrhotic patients. Non-cirrhotic patients may also develop bleeding during liver surgery. Moreover, cirrhotic patients have both increased risk of spontaneous bleeding as well as increased risk of developing thrombosis. In fact, the risk of thrombosis may be higher in these patients than the risk of a bleeding complication.<sup>17-19</sup> In addition, multiple compensatory changes for hemostatic defects may occur in patients with cirrhosis such as elevation of von Willebrand factor (VWF) and decreased levels of ADAMTS-13 that rebalance the thrombocytopenia and functional platelet defects.<sup>14</sup> In addition, defects in procoagulant proteins are compensated for by defects in anticoagulant proteins. Importantly, the conventional coagulation assays such as INR and APTT reflect only functionality of some of the pro-coagulant factors and are fully insensitive to anticoagulant proteins. Therefore conventional coagulation assays in patients with complex hemostatic abnormalities such as patients with cirrhosis are not reliable in predicting bleeding risk.

In chapter three, we evaluated the coagulation status of patients undergoing a (extended) right hemi-hepatectomy using both conventional coagulation tests and thrombin generation assays. Using the thrombin generation assay, we found that partial liver resection was associated with postoperative hypercoagulability, while substantially prolonged PT levels were detected in same patients, reflecting hypocoagulability. In

samples taken from hepatectomy patients, the thrombin generation potential remained consistently and significantly high after induction of thrombomodulin, the physiological activator of the natural anti-coagulant protein C. This reflects resistance to the anti-coagulant action of thrombomodulin that may be explained by the sustained post-operative deficiency of the natural anti-coagulant protein C and high levels of factor VIII. Compared to conventional coagulation assays that only reflect functionality of some of the pro-coagulant factors, the thrombin generation assay measures the total amount of thrombin generated during in vitro coagulation taking plasma concentrations of both pro- and anti-coagulants into account. Therefore, the thrombin generation test likely better reflects the in vivo hemostatic balance

compared to conventional coagulation tests. Our data suggests that a restrictive policy of plasma transfusion during liver resection and that even a more extensive anticoagulation prophylaxis might be warranted since the risk of developing thromboembolic events outweighs the risk of bleeding.<sup>20-22</sup> Clinical studies evaluating the policy of more extensive pharmacological anticoagulation prophylaxis in patients undergoing liver resection might be warranted.

Another key factor contributing to the outcome following liver surgery is the ability of the remnant liver to sustain the metabolic needs of the body and to regenerate.<sup>23</sup> Despite significant improvements in preoperative screening, a significant proportion of patients develop postoperative complications because the remnant liver or graft is too small or of poor quality to sustain sufficient organ function. This condition is defined as “small for size syndrome (SFSS)” that is characterized by coagulopathy, hypoalbuminemia, ascites, hyperbilirubinemia, encephalopathy, multi organ failure, and ultimately death.<sup>24</sup> Defective liver regeneration has been shown to be a key contributor for the development of SFSS.<sup>23-26</sup> Therefore, strategies to accelerate posthepatectomy liver regeneration and identification of patients at risk are needed.

Liver regeneration has been studied extensively, and the pathways involving liver regeneration are largely recognized.<sup>27-29</sup> Yet, clinically available strategies to enhance posthepatectomy liver regeneration are still lacking. Emerging evidence from in vitro models and small animal models suggest that platelets are an attractive target to accelerate posthepatectomy liver regeneration.<sup>30-36</sup> Platelets contain a wide range of biologically active molecules<sup>37,38</sup> that might contribute to the proliferation of hepatocytes following liver resection. In addition, recent innovative studies have shown that platelets are capable of *de novo* synthesis of a variety of proteins, even though platelets lack a nucleus. Platelets contain both pre-mRNA and mature mRNA and possess the machinery to splice pre-mRNA and to translate this into protein.<sup>39,40</sup> Given the recent medical advances in targeting platelets selectively,<sup>41</sup> platelets might be a promising target to enhance posthepatectomy liver regeneration and to reduce the risk of SFSS. Yet, it should be emphasized that the beneficial effect of endogenous platelets on liver regeneration and SFSS do not advocate transfusion of exogenous platelet concentrate since transfusion of platelets is associated with an increased risk of postoperative morbidity and mortality.<sup>3,42</sup>

The role of platelets in mediating liver regeneration has been subject of several small and large animal studies, and few clinical studies in humans.<sup>33-36,43</sup> Based on in vitro and animal studies, we evaluated the role of platelets in liver regeneration in a retrospective study of patients who underwent a partial liver resection for colorectal liver metastasis. We provided the first evidence that platelets may also contribute to liver regeneration in humans. Specifically, we showed that a low postoperative platelet count ( $< 100 \times 10^9/L$ ) after partial

liver resection was associated with an increased risk of mortality and delayed recovery of liver function. The platelet count was a strong and independent predictor of delayed recovery of liver function besides other established risk factor such as RBC transfusion (i.e., extent of bleeding) and the percentage of liver volume removed. Our findings suggest that platelets are likely target to accelerate posthepatectomy liver regeneration. Yet, because of the retrospective design of our study we cannot draw firm conclusions. However, if it is confirmed that platelets are directly related to liver regeneration, selective platelet targeting therapies or enhancement of platelet counts in patients undergoing a major liver resections or (partial) liver transplantation might be an option to prevent posthepatectomy liver dysfunction. Moreover, the clinical implementation of strategies to stimulate platelet-mediated liver regeneration may go beyond the context of partial liver resection or (partial) liver transplantation. It may also be applied in other clinical scenarios in which there is a demand for liver regeneration such as acute liver failure and liver fibrosis. Nevertheless, the precise mechanism by which platelets mediate liver regeneration have yet to be identified. A study from our group <sup>44</sup> showed that platelet internalization followed by platelet RNA transfer to the hepatocytes was in part responsible for platelet-induced hepatocyte proliferation in vitro. Inhibition of platelet uptake as well as treatment of platelets with an RNA-degrading enzyme significantly decreased in vitro proliferation of hepatocytes. It was hypothesized that functional transfer of either or both coding and regulatory RNA species from platelets to hepatocytes may be the key drivers of platelet-mediated liver regeneration. Meyer and colleagues <sup>45</sup> suggested that platelets are internalized either by liver endothelial cells or by hepatocytes from the liver sinusoids and the space of Disse following hemi-hepatectomy. Subsequently, they may release their bioactive contents such as serotonin and other growth factors that directly or indirectly stimulate liver regeneration.

Starlinger and colleagues<sup>43</sup> reported recently that a low preoperative serotonin level was associated with postoperative liver dysfunction and morbidity. They concluded to have found evidence that the platelet serotonin levels correlated with liver regeneration in humans. In chapter five we provide evidence against the notion that the decreased postoperative levels of serotonin were associated with liver regeneration. In fact, we prospectively studied the levels of circulating serotonin in patients undergoing a (extended) right hemi-hepatectomy, a pylorus preserving pancreaticoduodenectomy (PPPD), and healthy controls. In addition, we studied the levels of serotonin in afferent and efferent hepatic veins just prior to the start and just after the completion of parenchymal transection. Serotonin levels decreased postoperatively in both patient groups, but the decline was comparable between the hepatectomy and PPPD groups. We also found no significant change in serotonin levels between samples taken in the afferent and efferent liver veins prior to and after hemi-hepatectomy, suggesting that serotonin is not consumed within the regenerative liver.

Platelets are also increasingly recognized as important players in cancer growth and metastasis.<sup>46-51</sup> Elevation of the circulating platelet count (i.e., thrombocytosis),<sup>52-55</sup> increased platelet activation, hypercoagulability, and an increased risk of developing venous thromboembolism (VTE) have been reported in different types of cancer<sup>56-61</sup>. Elevation of the platelet count has also been reported in patients with HCC.<sup>54,55</sup> The elevation of platelet count in HCC is interesting for many reasons. First, almost 90% of patients who are diagnosed with HCC have underlying cirrhosis, which is associated with thrombocytopenia.<sup>62</sup> Second, cirrhotic patients have a relatively unstable hemostatic balance that is evidenced by the occurrence of bleeding and thrombotic complications in a significant proportion of patients.<sup>14</sup> If HCC, like other types of cancer, leads to a hyperactive primary hemostatic system, the development of HCC in a patient with cirrhosis could shift the balance towards thrombotic complications. Third, given the clinical benefit of aspirin and other platelet inhibitors in metastasis and death from colorectal cancer, the concept of activated platelet guided anti-cancer drug delivery has been suggested that may increase drug concentrations at the cancer sites, while reducing the systemic toxicities.<sup>61,63,64</sup> Based on these assumptions, we evaluated whether the development of HCC in patients with cirrhosis results in hyperactivity of primary hemostasis including elevation of platelet activation, altered VWF/ADMTS13 ratio, and platelet activatability. We found no difference in the activity of the hemostatic system in patients with and without HCC. We also found no significant difference in basal platelet activation between the patients and healthy controls. We, however, found decreased activatability in patients compared to controls. Our findings are in sharp contrast with studies in other types of cancer in which increased platelet activation was reported. Differences in methodology such as using soluble P-selectin or CD40 ligands as markers of in-vivo platelet activation instead of a more direct measure of platelet activation as used in our study may be responsible for these differences.<sup>65</sup> Nevertheless, our in vitro activation assay may not fully reflect physiology since platelet activation when studied under conditions of flow is similar in patients with cirrhosis compared to controls, despite decreased platelet activation capacity in assays performed under static conditions.<sup>66</sup> Based on our findings we suggest that the concept of activated platelet guided anti-cancer drug delivery may be less effective in cirrhotic patients with HCC compared to patients with other types of cancer. The changes in platelet function in patients with HCC appear fully driven by cirrhosis rather than cancer. This may have consequences for antihemostatic therapies, which need to be tailored to the cirrhosis rather than the presence of cancer. Whether the development of HCC results in hypercoagulability in non-cirrhotic patients needs further studies.

Elevation of angiogenic proteins has been documented in platelets of mice in presence of microscopic tumors (< 1 or 2 mm), and in platelets of patients with colorectal cancer<sup>67,68</sup>. As platelets store and release a number of angiogenic regulators, these molecules might function as a biomarker for the presence of cancer.<sup>47,69,70</sup> We studied if the levels of angiogenic proteins are altered in platelets of patients with hepatitis B- or C related cirrhosis



with HCC. We studied seven angiogenic proteins including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and platelet-derived growth factor (PDGF). We found no significant differences in platelet levels of any of these proteins between patients who did or did not have HCC. In contrast, we found significant difference in platelet levels of many of these proteins between the patients to healthy controls. This finding may again suggest that the cirrhosis rather than the cancer drives changes in platelet-derived angiogenic proteins. The clear absence of a difference between patients with or without HCC indicates that intraplatelet levels of angiogenic proteins may not be used as a biomarker for the presence of cancer in cirrhosis. Nevertheless, we cannot draw firm conclusions due to the small cohort size, heterogeneity of the patient population, relatively small cancer size and non-advance stage of HCC, and because the majority of HCC patients were not “treatment-naïve”. Whether the levels of angiogenic proteins in platelets of patients with HCC without cirrhosis alters, needs further evaluation.

#### **PREDICTORS OF OUTCOME FOLLOWING LIVER SURGERY**

While studying the outcome following liver surgery, we observed a paradoxical inverse behavior of immediate postoperative gamma glutamyl transferase (GGT) compared to other liver function parameters such as total bilirubin, AST, and ALT following liver resection and patients who underwent an operation for a ruptured abdominal aortic aneurysm<sup>71,72</sup>. The clinical relevance of GGT is currently limited to patients with cholestasis, as a liver damage maker after excessive alcohol intake, and as a predictor of usage of certain drugs.<sup>73</sup> Epidemiological studies in the general population correlate an elevated GGT with cancer development, overall cardiovascular mortality, hypertension, hypertriglyceridemia, obesity, type 2 diabetes mellitus, and stroke.<sup>74-82</sup> Yet, GGT is usually elevated in liver transplant recipients that experience good outcomes. Based on these observations, we studied the clinical relevance of GGT in liver transplant patients early- and late postoperatively. We found that the elevation of GGT during the first postoperative week was significantly associated with increased 90-day and 5-year survival. In contrast, an elevated GGT 6 month after transplantation was associated with significantly decreased 5-year survival. Subsequently, we studied the levels of GGT in critically ill patients admitted to the intensive care unit. We found that elevated GGT levels were inversely associated with in hospital mortality; the more deviated the levels of GGT the better the hospital survival and vice versa. GGT was not correlated with most of the liver function tests such as AST ( $r=0.11$ ), ALT ( $r=0.11$ ) and total bilirubin ( $r=0.17$ ). However, it did correlate with alkaline phosphatase ( $r=0.55$ ). Based on these observations, we suggest that postoperative increase in GGT may not simply be due to the release of GGT from damaged cells because GGT levels do not correlate with other liver damage makers. Besides, the rise in postoperative GGT occurs gradually within a week, reaching the maximum between day 7 and 9 postoperatively. It appears unlikely that GGT is



leaking from damaged cholangiocytes for a week and that patients who survive the hospital admission would leak more than those who did not survive the hospital admission.

Given the critical role of GGT in glutathione (GSH) metabolism, it seems plausible that GGT plays a key role in oxidative stress.<sup>73,83,84</sup> Studies on oxidative stress in patients with chronic hepatitis C virus infection showed an association between the levels of GGT and 8-hydroxydeoxyguanosine (8-OHdG), a marker of oxidative DNA damage. Patients who had a high level of 8-OHdG had significantly higher GGT levels but normal ALT levels.<sup>85</sup> Moreover, GGT-deficient mice showed an increase in oxidative stress in the kidney, accumulation of DNA damage in the organs, depletion of mitochondrial glutathione, and impaired production of ATP suggesting the lack of normal antioxidant defenses<sup>86-89</sup>. Administration of N-acetylcysteine was shown to reverse many diseases caused by GGT-depletion<sup>86-89</sup>. In humans, N-acetylcysteine is the treatment of choice in acetaminophen induced acute liver failure acting as a hepatoprotective agent by restoring hepatic glutathione.

These findings highlights the nature and significance of the normal physiological role of GGT in humans defense mechanism against the oxidative stress induced by reactive oxygen species (ROS). If it is true that GGT protects the hepatocytes against the oxidative stress, this might have clinical implications not only for the postoperative patients but also in patients with chronic and sustained hepatitis- or alcohol-related hepatotoxicity or patients with acute liver failure. Since elevated GGT, almost 100 fold that of normal levels, was found to have no cytotoxic or negative impact on humans health and survival,<sup>90</sup> GGT upregulation or administration might be an option. Yet, more research before this hypothesis can be tested in clinical practice is needed.

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