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

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# CHAPTER 1

## GENERAL INTRODUCTION AND RATIONALE OF THE THESIS

The liver is the largest internal organ in the body and it has several unique functions. These include synthesis of clotting factors involved in coagulation and fibrinolysis,<sup>1</sup> carbohydrate and lipid metabolism, detoxification, and bile production that is essential for absorption of fat and other lipophilic nutrients. A well-functioning liver is therefore pivotal for human's well-being and survival.<sup>2,3</sup> Acute and chronic liver failure represent major hurdles worldwide resulting over 1 million deaths a year.<sup>4,5</sup> In developing countries viral infections such as hepatitis A, B, and E are the predominant causes of liver disease.<sup>6,7</sup> In contrast, the most common etiologies of cirrhosis in western societies are hepatitis C virus infection (HCV), excessive alcohol intake, genetic and inherited disorders, autoimmune conditions, and non-alcoholic fatty liver diseases (NAFLD).<sup>5,8</sup> Although cirrhotic patients can be asymptomatic, progressive deterioration may lead to the development of end stage liver disease (ESLD). ESLD is associated with severe complications including portal hypertension, ascites, hepatic encephalopathy,<sup>5</sup> and development of hepatocellular carcinoma (HCC). HCC has a 5-year cumulative risk of 15% and 10% in endemic areas and developed countries, respectively.<sup>9,10</sup> In fact, HCC is responsible for 50% to 70% of liver-related mortality in patients with compensated cirrhosis.<sup>11,12</sup>

Despite medical advances, partial liver resection and liver transplantation are the only curative options for patients with end stage acute or chronic liver failure, or cancer of the liver. However, postoperative complications still occur in many patients<sup>13-15</sup> because the liver remnant or grafts are too small or of poor quality to maintain the synthetic, excretory and detoxifying functions of the liver.<sup>16,17</sup> Consequently, multiple organ failure, sepsis, and death can occur within days after surgery<sup>17,18</sup> unless prompt and sufficient liver regeneration occurs.

Liver regeneration starts immediately following partial liver resection.<sup>19</sup> Hepatocyte proliferation terminates within days in humans.<sup>3</sup> However, Kele and associates<sup>20</sup> showed that the remnant liver did not regenerate to its preoperative total liver volume after six months of surgery. The mechanism of liver regeneration has been studied extensively,<sup>21</sup> yet little is known about factors promoting tissue repair and liver regeneration in humans following surgical procedures involving the liver.<sup>21,22</sup> Studies in rodents such as mice show that blood platelets play a pivotal role in liver regeneration.<sup>23-26</sup> Induction of thrombocytosis by administration of a single dose of thrombopoietin or by splenectomy promoted liver regeneration.<sup>23</sup> Conversely, antibody-induced depletion of platelets and inhibition of platelet function using clopidogrel, a P2Y<sub>12</sub> receptor blocker significantly reduced proliferation of hepatocytes.<sup>23-26</sup> Also, transfusion of platelets or platelet-rich plasma promotes liver regeneration in mice.<sup>27,28</sup>

Although the exact mechanism of platelet-mediated liver regeneration is yet to be determined, there is evidence that platelet derived growth factors and serotonin, which are

stored within the platelet granules, play a key role in proliferation of hepatocytes.<sup>26,29</sup> Nevertheless, the role of platelets and bioactive molecules released by platelets in regeneration in humans is incompletely understood.

#### HEMOSTASIS IN PATIENTS WITH CIRRHOSIS AND HEPATOCELLULAR CARCINOMA

Given the critical role of liver in coagulation, fibrinolysis, and platelet metabolism,<sup>1</sup> patients with ESLD frequently have substantial abnormalities in their hemostatic system. Conventional coagulation assays such as the prothrombin time (PT) / international normalized ratio (INR) and activated partial thromboplastin time (APTT) are very often prolonged in patients with liver disorders reflecting hypocoagulability in these patients.<sup>30</sup> However, conventional coagulation tests are poor predictors of bleeding in patients with liver disease.<sup>30,31</sup> In fact, PT and APTT assays may not accurately reflect the hemostatic status since these assays only measure the pro-coagulant activity and ignore the natural anti-coagulant systems.<sup>30,32</sup> Importantly, many centers tend to correct these *in vitro* measures prophylactically prior to or during invasive procedures, exposing the patients to transfusion-related risks.<sup>33-35</sup> In addition, the incorrect assumption that patients with liver disease are “auto-anticoagulated” is (partly) based on wrongful interpretation of routine hemostasis tests.<sup>36,37</sup> In fact, patients with ESLD are at risk for both bleeding and thrombotic complications.

Although cirrhotic patients commonly have a decreased platelet count,<sup>38,39</sup> the development of HCC in these patients is associated with elevated platelet count.<sup>40-42</sup> Given the interaction between the cancer and platelets and the increased risk of VTE in patients with HCC,<sup>44,45</sup> elevated platelet counts in cirrhotic patients in the presence of HCC might be considered a paraneoplastic manifestation of HCC.<sup>41,43</sup> In fact, thrombotic complications are a major cause of death in patients with cancer.<sup>46</sup> Yet, little is known about the interaction between the platelets and HCC in the presence of cirrhosis.<sup>47</sup> In animal models, it has been shown that circulating platelets take up and sequester angiogenesis regulators in the presence of a microscopical (<1mm) human tumor.<sup>48</sup> A recent study in humans showed that angiogenic proteins were elevated in platelets from patients with colorectal cancer,<sup>49</sup> and platelet levels of these proteins were independent predictors of the presence of a malignancy. It has been established that platelet-induced angiogenic molecules are involved in extra-hemostatic platelet effects such as liver regeneration, tumor development and progression, and inflammation.<sup>50</sup> However, it is not clear if the development of HCC in cirrhotic patients affects platelet biology or the levels of bioactive molecules that are stored within platelet granules.

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

After liver surgery, all liver function tests (LFTs) may be abnormal including aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, albumin, antithrombin, PT / INR, and gamma-glutamyltransfrase (GGT).<sup>51,52</sup> Hence, LFT derangements may reflect physiologic changes rather than pathologic conditions following procedures involving the liver. Nevertheless, LFTs may predict morbidity and mortality beyond a certain threshold value at a certain time point following liver surgery.<sup>15,53</sup> As such, total bilirubin, PT/INR, albumin, antithrombin (AT), AST, ALT, and clinical signs such as ascites and encephalopathy have been consistently used in the literature to predict outcomes following partial liver resection and liver transplantation.<sup>15,17,53</sup> Also blood platelet number decreases transiently following procedures involving the liver. Given the key role of platelets in liver regeneration,<sup>26,54</sup> immediate postoperative platelet count may accurately predict postoperative outcomes.

Contrary to other LFTs, an elevated (i.e., more deviated from the normal) GGT has shown to be associated with an improved hospital survival following surgical correction of a ruptured abdominal aortic aneurysm.<sup>55</sup> However, in the general population, a chronically elevated GGT is associated with an elevated all-cause mortality related to cardiovascular disease, metabolic syndrome and cancer.<sup>56-59</sup> GGT is a sensitive test of liver disease regardless of its causes, but it lacks specificity.<sup>60,61</sup> GGT increases following procedures that involve the liver, and it has been suggested as a biomarker of biliary epithelial injury.<sup>62</sup> Yet, little is known about the clinical relevance of elevated GGT following liver surgery.

## **AIMS OF THE THESIS**

This thesis consists of clinical and pre-clinical research that aims to gain better understanding of factors influencing the outcome of liver surgery and the paradoxical role of GGT following liver transplantation and surgery. In addition, two studies evaluate the impact of HCC development on hemostasis in cirrhotic patients.

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