Assessing outcomes of liver surgery : current status and future prospects

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Chapter 1

Liver failure after partial hepatic resection – A general introduction to the clinical problem

Adapted from:

van den Broek MA, Olde Damink SW, Dejong CH, Lang H, Malagó M, Jalan R, Saner FH. Liver failure after partial hepatic resection: definition, pathophysiology, risk factors and treatment. *Liver Int* 2008; 28: 767-80.

ABSTRACT

Post-resectional liver failure (PLF) is a life-threatening complication that may arise after partial hepatic resection. In this chapter, the definition, incidence rate, pathogenesis, risk factors, risk assessment, prevention, clinical features, treatment and economic impact of PLF will be reviewed.

The reported incidence rates of PLF vary widely due to the lack of a uniform definition. Leaving the extremes out of consideration, the incidence rate of PLF ranges between 1 and 9 per cent. It is the leading cause of death after partial liver resection. An inadequate quantity or quality of residual liver mass are key events in the pathogenesis of PLF. Risk factors are either surgery or patient-related and include extensive resection of functional liver leaving small remnant liver mass and/or impaired liver function due to underlying liver disease. It is essential to identify patients at risk of PLF during the preoperative assessment, which includes evaluation of liver volume, anatomy and function. Preventive measures should be applied whenever possible as curative treatment options for PLF are limited. These preventive measures intend to increase remnant liver volume and protect remnant liver function. Patients suffering from PLF often develop multi-organ failure. Management principles focus upon support of end-organ and liver function. Due to the complex nature of the disease, the economic burden of PLF is high.

Partial hepatic resection is the ultimate treatment for various benign and malignant liver tumours. In the last decade, outcomes of liver surgery have considerably improved, making a partial hepatic resection a relatively safe procedure[1, 2]. The fact that partial hepatectomy is used in the setting of living donor liver transplantation as an accepted alternative to cadaveric donor liver transplantation may serve as an example[3, 4].

The improved safety of liver surgery led to broadening of the indications for partial hepatic resection towards more extensive resections in high-risk patient groups. The resultant small remnant liver volume and pre-existent impaired liver function increase the risk of post-resectional liver failure (PLF). PLF is associated with major postoperative morbidity and mortality[5, 6]. In this chapter, the definition, incidence rate, pathogenesis, risk factors, risk assessment, prevention, clinical features, treatment and economic impact of PLF will be reviewed.

DEFINITION

Up until recently, there was no uniformity concerning the definition of PLF, which made it difficult to interpret and compare results of clinical trials. In general, PLF was characterized as failure of one or more of the hepatic synthetic, detoxifying and excretory functions resulting in hypoalbuminemia, prolonged prothrombin time, hyperbilirubinemia, elevated serum lactate and/or hyperammonemia[7-10]. In 2011, the International Study Group of Liver Surgery proposed a standard definition of PLF (Table 1.1)[11]. This definition has been evaluated in a large patient cohort, but prospective, external validation is warranted.

Table 1.1 Consensus definition and severity grading of post-resectional liver failure by the International Study Group of Liver Surgery.

Definition	A postoperatively acquired deterioration in the ability of the liver (in patients with normal and abnormal liver function) to maintain its synthetic, excretory, and detoxifying functions, characterized by an increased INR (or need for clotting factors to maintain a normal INR) and hyperbilirubinemia (according to the normal cutoff levels defined by the local laboratory) on or after postoperative day 5. If INR or serum bilirubin is increased preoperatively, PLF is defined by an increasing INR (decreasing prothrombin index) and increasing serum bilirubin concentration on or after postoperative day 5 (compared with the values of the previous day). Other obvious causes for the observed biochemical and clinical alterations such as biliary obstruction should be excluded
Grade A	PLF resulting in abnormal laboratory parameters but requiring no change in the clinical management of the patient
Grade B	PLF resulting in a deviation from the regular clinical management but manageable without invasive treatment
Grade C	PLF resulting in a deviation from the regular clinical management and requiring invasive treatment

INR, international standardized ratio; PLF, post-resectional liver failure.

In addition to this qualitative definition, PLF is quantitatively reasonably well defined by the so-called 50-50 criteria designed by Balzan and colleagues. In these 50-50 criteria, PLF is described as a prothrombin index below 50 per cent [equal to an international standardized ratio > 1.7] and serum bilirubin level of more than 50 µmol/L [2.9 mg/dL] on the 5th postoperative day (POD)[8]. If the 50-50 criteria were met, patients had a significantly increased risk of mortality after liver surgery. A prospective follow-up study showed that these 50-50 criteria could serve as an independent prognostic factor of liver surgery-related death as early as POD 3[6]. Another group identified peak bilirubin above 120 µmol/L [7.0 mg/dL] as a simple, sensitive and specific cutoff value for the prediction of PLF-related death[10].

All definitions of PLF are open to discussion. Their major drawback is that they cannot be applied in the early postoperative phase, when interventions that aim at preventing PLF may be most beneficial. Their shortcomings could be overcome by the prospective development of new definitions comprising sensitive biomarkers such as indocyanine green or ^{13}C-methacetin kinetics[12, 13].

INCIDENCE RATE

The incidence of PLF in a case mix of patients ranges anywhere between 0 and 32 per cent (based on [1, 2, 7, 8, 10, 14-29]). The highest incidence rates have been reported in specific groups, such as patients with cirrhosis of the liver, chemotherapy-associated hepatotoxicity or steatohepatitis[30-33]. Leaving the extremes out of consideration, its incidence varies between 1 and 9 per cent. The mortality rate in patients suffering from PLF is high[10, 33]. Although the cause of death after partial hepatic resection is multifactorial, PLF seems to be the principal cause in 60 to 75 per cent of patients with lethal outcome[10, 19, 33, 34].

PATHOGENESIS

The resection of various amounts of functional liver mass induces both death and regeneration of remaining hepatocytes. Physiologically, regeneration outweighs hepatocyte death and both liver mass and function are rapidly restored[35-37]. For example, during the first ten days after right hepatectomy for living donor liver transplantation, liver mass restored from 39 up to 74 per cent of initial volume and liver biochemistry normalized rapidly[35]. This regeneration is triggered by an increased metabolic demand placed upon remnant hepatocytes (see for review [38]).

The pathogenesis of PLF is poorly understood and is thought to be related to an inadequate quality and/or quantity of remnant liver mass. The ability of the liver remnant

to surmount the effect of surgical resection depends on its capacity to limit hepatocyte death, enhance its regenerative power, and resist metabolic stress while preserving an adequate function[39, 40]. A variety of events can interfere with these protective processes. These events include hepatic parenchymal congestion, ischemia-reperfusion injury and postoperative infection[41-43].

Hepatic parenchymal congestion

Partial hepatic resection leads to a relatively augmented sinusoidal perfusion, inducing shear-stress and congestion of hepatic parenchyma[42]. This results in vascular and parenchymal damage similar to the small-for-size syndrome after liver transplantation, although less severe[44]. Inadequate venous drainage of the liver remnant might additionally induce hepatic venous congestion and functional hepatic volume loss[45].

Hepatic ischemia-reperfusion injury

Hepatic ischemia-reperfusion injury follows massive bleeding or hepatic in- and outflow occlusion during liver surgery. Although the resistance of the liver to warm ischemia is relatively high, hepatic ischemia and reperfusion activate a complex cascade that triggers the innate immune response and leads to cell death (see for review [41]). Although these processes are primarily intended to maintain homeostasis, unrestrained activation may become destructive.

Infection

Infection complicates the course of PLF either as a precipitant or during later stages[9, 33]. Schindl and colleagues showed that the hepatic phagocytosis capacity decreased after partial liver resection with an S-shape correlation to the extent of hepatic resection[43]. Diminished hepatic clearance of bacteria after resection of large amounts of functional liver mass combined with an increased bacterial load following either bacterial translocation after inflow occlusion or intra-abdominal abscesses, enhance the susceptibility for the development of infection and PLF[46].

RISK FACTORS

The most important, independent risk factors of PLF can be categorized as surgery or patient-related (Table 1.2). These factors include extensive liver resection, excessive intra-operative blood loss, prolonged operating time, male sex and advanced age[1, 5, 9, 10, 18, 19]. Also co-morbid conditions like diabetes mellitus as well as pre-existent liver diseases, such as steatohepatitis, cirrhosis, or chemotherapy-associated hepatotoxicity, predispose for the development of PLF[31, 47-50]. At present, it is unclear if extra-hepatic

Table 1.2 Risk factors of post-resectional liver failure.

Surgery-related
Extensive liver resection
Excessive intra-operative blood loss
Ischemia-reperfusion injury
Parenchymal congestion
Patient-related
Pre-existent liver disease • Cirrhosis • Steatosis and steatohepatitis • Chemotherapy-associated hepatotoxicity
Male sex
Advanced age (≥ 65 years)

procedures, like concomitant biliary or vascular reconstructions, influence the incidence of PLF individually or by their association with extended liver resection, increased operating time and/or excessive blood loss.

Surgery-related risk factors

Extensive liver resection

The number of hepatic segments resected significantly correlates with postoperative complication rates (odds ratio (OR) 1.2 (95 per cent CI 1.12 to 1.29))[19]. Remnant liver volume (RLV), defined as percentage remaining functional liver volume compared to preoperative functional liver volume, is regarded a reliable parameter to predict PLF and death, even more than the anatomic extent of the resection[9, 30, 51]. However, the exact amount of residual liver mass required to preserve sufficient liver function is unknown. In general, an RLV equal to or above 25 to 30 per cent in otherwise healthy livers is consistent with good post-resectional outcome[5, 9, 52]. An RLV below 25 per cent in normal livers predicted PLF with a positive predictive value of 90 per cent (95 per cent CI 68 to 99 per cent) and a specificity of 98 per cent (95 per cent CI 92 to 100 per cent)[5]. When liver function is restricted, such as in patients with cirrhosis or chemotherapy-associated hepatotoxicity, RLV should be as high as 40 per cent to increase the likelihood of adequate remnant liver function[18, 52-54].

Excessive intra-operative blood loss and blood transfusion

Intra-operative blood loss and the need for blood transfusion predispose patients to PLF (OR 4.17 (95 per cent CI 1.04 to 17.5))[18, 19, 55]. The cutoff point for an adverse outcome is suggested to be above 1 000 – 1 250 mL blood loss. Excessive blood loss leads to massive fluid shifts, which may induce bacterial translocation and systemic inflammation[56].

Massive bleeding also results in severe coagulopathy, which may predispose for intra-abdominal hematoma and subsequent infection.

Patient-related risk factors

Male sex

Male sex doubles the propensity for developing PLF and post-resectional morbidity (OR 1.98 (95 per cent CI not given)), consistent with earlier observations that males are more susceptible to develop postoperative complications[5, 10]. Hypothetically, circulating sex hormones are responsible as testosterone is thought to exert an immunodepressive effect while estrogens exert an immunoprotective effect[57].

Advanced age

Although data in literature are conflicting, advanced age (above 65 years) seems to predispose for PLF and post-resectional mortality (OR 1.8 (95 per cent CI 0.78 to 4.19))[8], especially after extended hepatic resections[10, 20, 58]. Elderly patients frequently suffer from co-morbid conditions and have a reduced regenerative capacity of hepatocytes[59, 60].

Steatosis and steatohepatitis

Subjects with elevated body mass index (above 30 kg/m^2) and subjects suffering from nonalcoholic fatty liver disease (NAFLD) experience significantly more complications after liver surgery compared to controls[31, 61-63]. Patients with steatohepatitis, but not with simple hepatic steatosis, have a higher incidence of post-resectional hepatic decompensation compared to patients with healthy livers (16.7 versus 6.9 per cent, p=0.049)[63]. The presence of NAFLD is hypothesized to be associated with an impaired hepatic microcirculation[64], decreased resistance to ischemia-reperfusion injury, increased intrahepatic oxidative stress and dysfunction in mitochondrial ATP-synthesis[65]. Animal studies report an impaired regeneration of steatotic livers, but the sparse clinical data currently available from living donors suffering from mild hepatic steatosis do not support these findings[66, 67].

Cirrhosis

The incidence of PLF after partial hepatic resection in patients with cirrhosis ranges between 5 and 10 per cent taking into account a higher number of restrictive surgical procedures performed in this subgroup[30, 47, 49]. The high risk of developing PLF in patients with cirrhosis can be explained by the wide range of co-morbid conditions like portal hypertension[68], diabetes mellitus, jaundice[69], malnutrition, hypersplenism and coagulopathy as well as frequently impaired preoperative liver function and hepatic functional reserve[70]. Furthermore, patients with cirrhosis seem to have an impaired hepatic regenerative capacity[37].

Chemotherapy-associated hepatotoxicity

Perioperative, systemic chemotherapy regimens improve progression-free survival in patients with colorectal liver metastases (CLMs) and have therefore become standard of care in this patient group[71]. However, they are associated with an increased incidence of PLF and PLF-related death as a result of chemotherapy-associated hepatotoxicity[32, 71-73]. Oxaliplatin induces sinusoidal injury of the non-tumour-bearing liver (the so-called sinusoidal obstruction syndrome (SOS)) in approximately 3 out of 5 patients[74, 75]. This sinusoidal injury is thought to reduce the regenerative capacity of the liver remnant[76, 77]. Treatment with irinotecan is associated with an increased risk of steatohepatitis (so-called chemotherapy associated steatohepatitis (CASH)), which increases 90-day mortality[32, 78].

RISK ASSESSMENT

Preoperative risk assessment should ideally consist of four features including clinical, biochemical, volumetric and functional data (see for review [79]). An assessment focussing on only one of these aspects is regarded not to be useful. A thorough evaluation of risk factors is generally believed to enable the selection of candidates suitable for a safe partial hepatic resection with low risk of PLF. However, the onset of PLF will remain unpredictable in a subset of patients.

Assessment of clinical condition

The identification of co-morbid conditions like obesity, diabetes mellitus, malnutrition, cardiovascular, pulmonary, hepatic or renal disease is pivotal as they increase the susceptibility for major complications, even if hepatic functional reserve is adequate[7, 50]. Additionally, the existence of portal hypertension must be objectified, as this elevates the risk of bleeding and PLF[68].

Assessment of biochemical parameters

Tests analyzing hepatic synthetic (serum albumin and clotting factors) or excretory function (serum bilirubin) are non-specific for the assessment of hepatic function and do not correlate with post-resectional outcomes; however, they may indicate hepatic dysfunction[70, 80]. Furthermore, serum activities of transaminases as well as alkaline phosphatase and γ-glutamyl-transferase are non-specific for the evaluation of hepatic function, but can signal hepatocyte necrosis, increased hepatitic activity or the presence of cholestasis.

Assessment of liver anatomy and volumetry

Standard liver resection planning is based on contrast-enhanced 2D computed tomography (CT) or magnetic resonance imaging (MRI), supplemented with intra-operative ultrasonography. Based on these imaging techniques, information on the anatomy of liver segments, biliary structures, hepatic vasculature and tumour localization can be extracted. However, 2D CT supplies only marginal data about the distribution pattern of hepatic venous in- and outflow related to hepatic segments and tumour location[81]. In this context, 3D CT reconstructions have proven to deliver useful additional information in selected patients, for example those requiring an extended hepatic resection[82, 83].

Ultrasonography, CT and MRI are also able to provide information on the quality of hepatic parenchyma in patients with suspected underlying liver disease, such as hepatic steatosis or chemotherapy-associated hepatotoxicity[84, 85]. When pre-existent liver disease is expected and imaging studies are inconclusive, a (transjugular) biopsy of the non-tumorous liver or even a diagnostic laparoscopy may be helpful to establish the diagnosis.

Volumetric analysis can be performed using CT imaging, which provides good quality data on total, functional (i.e. total liver volume minus tumour volume) and remnant liver volume[86, 87]. Appropriate formulas combining body surface area and weight are available for the calculation of total liver volume and these formulas are hypothesized to reflect the metabolic demands more exactly than CT volumetry alone[88].

Assessment of liver function

Assessment of liver function is useful to determine hepatic functional reserve. Several dynamic tests quantitatively evaluate liver function, among which the indocyanine green retention at 15 minutes post intravenous injection (ICGR15), the galactose elimination test, the lidocaine-monoethylglycine-xylidide test (MEGX), the ^{13}C-methacetin test (LiMAx) and the ^{14}C-aminopyrine breath test are most frequently used. They all assess hepatic clearance or conversion of xenobiotics.

ICGR15 depends upon hepatic perfusion rate and subjects with an ICGR15 above 15 to 20 per cent are generally believed to have an impaired hepatic functional reserve[23, 47, 89]. Recently, ICGR15 above 10 per cent was found to be a reliable predictor for the presence of sinusoidal injury secondary to neoadjuvant chemotherapy treatment in patients with CLMs[72].

The hepatic cytosolic capacity is reflected by the galactose elimination test and the critical value is considered to be elimination of less than 6 mg/min/kg in patients without and less than 4 mg/min/kg in patients with hepatocellular carcinoma[90].

The MEGX test, LiMAx test and ^{14}C-aminopyrine breath test are based on the rate of metabolite formation of specific drugs. MEGX and LiMAx tests both reflect the conversion rate of a prodrug by hepatic cytochrome P450. For MEGX, a value equal to or below

25 µg/L was related to PLF in patients with cirrhosis[91]. A LiMAx cutoff value of 85 µg/kg/hr on POD1 had a sensitivity of 100 per cent and specificity of 94 per cent for identifying patients developing PLF[12]. Finally, the aminopyrine breath test evaluates the hepatic oxidative function by measurement of $^{14}CO_2$ exhalation. The normal value is an exhalation of 7 per cent $^{14}CO_2$ and the critical value seems to be below 2.3 per cent[92, 93]. There is no consensus regarding the validity of a single test for assessment of liver function and hepatic functional reserve in operative planning.

Assessment of liver function in patients with cirrhosis

For patients with cirrhosis, scoring systems used to assess the feasibility of partial hepatic resection are the Child-Pugh score and the model for end-stage liver disease (MELD) score[7, 94, 95]. As they both are designed for other purposes, their validity to predict post-resectional outcome has only recently been established and results are inconsistent. In general, Child-Pugh class C is considered an absolute contra-indication for surgery and class B permits only minor liver resection[96]. Schroeder and colleagues reported superiority of the Child-Pugh to MELD score in predicting short-term morbidity and mortality after partial hepatic resection[7]. However, other authors stated that preoperative MELD score was a highly reliable predictor in certain subgroups. For example, a MELD score above 11 in patients with cirrhosis could predict PLF accurately (area under receiver operating characteristic curve 0.92 (95 per cent CI 0.87 to 0.96))[97].

PREVENTION

As curative treatment options for PLF are limited, prevention of this life-threatening condition is essential.

Surgery-related risk factors

Extensive liver resection

Portal vein occlusion and/or two-stage hepatectomy can be considered in case of expected small RLV. Portal vein occlusion by either embolization or ligation of one of the portal veins is advised in patients with normal liver function if RLV is estimated to be below 25 to 30 per cent or in patients with impaired liver function (reflected by an IGCR15 between 15 to 20 per cent) and estimated RLV below 40 to 45 per cent[98-100]. Its effectiveness depends on the severity of pre-existent liver disease and co-morbid conditions, ranging from 20 to 46 per cent volume increase after 2 to 4 weeks[77, 99, 100]. Portal vein embolization increased the feasibility of partial hepatectomy by 19 per cent[99], and had a complication rate between 9 to 13 per cent (see for review [98]). It is hypothesized

to promote intra-hepatic tumour growth in the non-embolized liver, but this does not seem to have an effect on long-term outcomes of partial hepatic resection for CLMs[101]. Interval chemotherapy might be beneficial, though, seems to negatively affect hypertrophy ratio[77]. A combination of portal vein occlusion and in situ split liver transection with preservation of arterial inflow and venous outflow (the ALPPS approach) has recently been proven to induce a 40 to 80 per cent hypertrophy of the future liver remnant in only 6 days, which was remarkably greater than the hypertrophy observed by portal vein occlusion alone[102]. Stem cell therapy as adjunct to portal vein occlusion seems promising, but robust clinical evidence for its effectiveness is lacking to date[103].

Two-stage hepatectomy utilizes the regenerative capacity of the liver. Studies focussing on the feasibility of two-stage hepatectomy combined with neoadjuvant chemotherapy and/or portal vein occlusion reported success rates of 70 to 81 per cent along with an increase in median survival time compared to palliative chemotherapy alone in patients with initially unresectable CLMs[77, 104, 105] (see for review [106]).

Excessive intra-operative blood loss and blood transfusion

Lowering central venous pressure during dissection to below 5 cmH_2O limits intra-operative blood loss without deterioration of renal function[107, 108]. A combination of the former with portal triad clamping or total vascular exclusion is most advantageous for the prevention of excessive intra-operative blood loss[109]. The latter procedures both induce hepatic ischemia-reperfusion injury, but total vascular exclusion leads to more important hemodynamic changes and higher complication rates compared to hepatic inflow occlusion alone[110]. With respect to hepatic inflow occlusion, intermittent portal triad clamping is regarded superior to continuous clamping[111]. However, the optimal ischemic intervals are unknown. Ischemic preconditioning (IPC) before portal triad clamping reduces hepatocyte damage in a murine as well as a human model of hepatic inflow occlusion[65, 112-115]. Petrowsky and colleagues reported that IPC combined with continuous portal triad clamping was as effective as intermittent clamping in non-cirrhotic livers, though intermittent clamping was accompanied by significantly higher intra-operative blood loss, transfusion requirement and operating time[116]. The latter study also showed that the protective effect of IPC seemed to diminish in patients above 65 years of age. Consequently, intermittent clamping is suggested to be superior to IPC combined with continuous clamping in elderly patients[116].

Patient-related risk factors

Steatosis and steatohepatitis

Data from living liver donors suffering from biopsy-proven moderate steatosis revealed that a body weight reduction of 5 per cent or intervention with low-fat, high-protein

diet and exercise significantly improved hepatic steatosis[117, 118]. In addition, drugs that are able to reverse NAFLD have become available recently[119]. The beneficial effects of preoperative voluntary weight loss or drug therapy on post-resectional outcomes in patients with NAFLD scheduled to undergo liver surgery have not been studied to date.

Cirrhosis

Approximately 65 to 90 per cent of patients with advanced liver disease suffer from protein-calorie malnutrition[120]. It has therefore been hypothesized that the nutritional status of these patients should be corrected *prior* to surgery. Indeed, a beneficial effect of additional parenteral nutrition has been demonstrated in patients with cirrhosis undergoing major hepatectomy[121].

Chemotherapy-associated hepatotoxicity

Optimizing the interval between neoadjuvant chemotherapy treatment and hepatic resection might reduce the risk of postoperative complications, as a longer interval between chemotherapy and surgical resection seemed to decrease the incidence of sinusoidal injury[72, 122]. Addition of bevacizumab, a monoclonal antibody against vascular endothelial growth factor, to conventional chemotherapeutic agents also seemed to protect against the development of SOS in patients with CLMs, as did oral aspirin treatment[123-125].

MANIFESTATION

The majority of patients with PLF do not only suffer from liver failure, but also meet the criteria for the systemic inflammatory response syndrome and multi-organ failure.

Liver

The clinical consequences of PLF are jaundice, coagulopathy, ascites, oedema and/or hepatic encephalopathy (HE). Data on liver function on different days after uncomplicated hepatic resection showed temporal liver dysfunction reflected by initially increased serum bilirubin levels and increased prothrombin times before normalization of these values on POD7-10[8, 35, 36]. However, when prothrombin index dropped below 50 per cent and serum bilirubin level exceeded 50 µmol/L [2.9 mg/dL] on either POD3 or POD5, the risk of PLF and PLF-related mortality increased significantly[6, 8].

Circulation

Circulatory failure occurring during PLF resembles the circulatory failure of patients with sepsis[126]. The pathophysiological changes usually observed are enhanced vascular

permeability, diffuse intravascular coagulation and peripheral vasodilatation, which are clinically represented by reduced peripheral resistance leading to hemodynamic instability.

Lungs

Although moderate pulmonary oedema is a normal finding after partial hepatic resection due to general hemodynamic alterations, this does usually not impair oxygen exchange[127]. Severe remote lung injury, pulmonary oedema and acute respiratory distress syndrome can develop as part of multi-organ failure accompanying PLF.

Kidneys

Post-resectional renal dysfunction can either result from perioperative disturbances in renal circulation inducing acute tubular necrosis or can accompany PLF[107, 128]. It is characterized by an increase in creatinine level, uremia or oliguria and may cause ascites formation, pleural effusion and fluid overload requiring diuretics or hemofiltration[129]. There is a distinct chance of reversibility of renal failure when there is recovery of PLF. It can be hypothesized that renal failure interferes with the pivotal role of the kidney in ammonia excretion, leading to hyperammonemia and HE in patients suffering from PLF[130].

Brain

Patients with PLF may suffer from HE which is a potentially reversible neuropsychiatric disorder characterized by varying degrees of confusion and disorientation[131]. Hyperammonemia plays a central role in the development of HE[132]. There seems to be a strong association between infection and the development of HE, as inflammation makes the brain more vulnerable to ammonia[133]. Data concerning the incidence of HE after partial hepatic resection are sparse.

TREATMENT

Although the mortality rate of PLF is high, it is a potentially reversible disorder because of the regenerative capacity of the liver remnant. Large, randomized controlled trials (RCTs) concerning the treatment of PLF are lacking and therefore, recommendations for its treatment based on high level of evidence research are difficult to make. Management principles resemble those applied to patients with acute liver failure, acute-on-chronic liver failure and sepsis and focus on support of end-organ and liver function[134, 135]. In desperate situations, rescue hepatectomy and subsequent liver transplantation may be considered.

Support of end-organ function

Goal-directed therapy should be provided for circulatory disturbances, renal and pulmonary dysfunction, coagulopathy, malnutrition and HE (Table 1.3). As there seems to be a strong link between infection and PLF, frequent cultures for bacteria and fungi are important[33]. The use of prophylactic antibiotics after partial hepatectomy for the prevention of infectious complications is not supported by evidence from literature[136, 137]. However, the administration of antibiotics in patients suffering from acute liver failure unrelated to surgery is associated with a significant decrease in infectious complications and this may also be advantageous in patients at high risk of PLF[138].

Table 1.3 Treatment goals of patients suffering from post-resectional liver failure.

System	Goal
Circulation	Central venous pressure between 8-12 mmHg Mean arterial pressure between 65-90 mmHg Hematocrit ≥ 30 per cent Pulmonary capillary wedge pressure ≤ 12-15 mmHg
Kidneys	Urine output ≥ 0.5 mL/kg/hr
Lungs	Arterial oxygen saturation ≥ 93 per cent Central venous oxygen saturation ≥ 70 per cent
Brain	Improvement of HE to grade ≤ 2
Coagulation	In case of bleeding: - Platelet count ≥ 50 10^9/L - INR ≤ 1.5
Nutrition	Enteral energy supply of 2000 kcal/day

HE, hepatic encephalopathy; INR, international standardized ratio.

Support of liver function

Support of liver function can be achieved by cell-based therapy or extracorporeal liver support systems. The rationale of cell therapy is to replace defective cells in order to substitute organ function. The use of hepatocyte or stem cell transplantation for the treatment of PLF seems promising, but scientific evidence for its effectiveness in man is lacking to date[139].

Extracorporeal liver support systems aim to replace the detoxifying or synthetic functions of the failing liver. A recent meta-analysis demonstrated a survival advantage of extracorporeal liver support devices in patients suffering from acute liver failure unrelated to surgery[140]. RCTs analyzing the effect of extracorporeal liver support systems in patients with PLF are lacking, but some non-controlled studies have been conducted on its effectiveness.

Plasma exchange

Plasma exchange is an extracorporeal supportive procedure in which plasma is separated from blood cells and treated or substituted with fresh frozen plasma. This technique supplies plasma components that are lacking (e.g. albumin and clotting factors) and removes water-soluble toxins related to HE (e.g. ammonia) hereby improving the clinical condition of patients suffering from PLF[141, 142].

MARS®

The molecular absorbent recirculating system (MARS®, Gambro, Lund, Sweden) removes water-soluble along with albumin-bound toxins from the plasma by means of dialyzing blood against an albumin-containing dialysate across an albumin-impregnated membrane[143, 144]. Promising results have been shown when applied during acute liver failure or acute-on-chronic liver failure[145], but the use of MARS for treatment of PLF has only been validated in small, uncontrolled and non-randomized trials. Unfortunately, MARS treatment for PLF and progressive septic multi-organ failure did not positively affect patient survival[146-148].

Prometheus®

Prometheus® (Fresenius Medical Care, St Wendel, Germany) uses the principle of fractionated plasma separation and adsorption for removal of water-soluble along with albumin-bound toxins. Albumin-bound toxins pass an albumin-permeable membrane and native albumin is subsequently detoxified by adsorption, after which the cleansed albumin is returned to the patient[143, 144]. The detoxifying capacity of Prometheus appeared to be superior to that of MARS when applied during acute-on-chronic liver failure[149], but no clinical survival benefit has been proven yet. Studies on the application of Prometheus for PLF are lacking.

Bioartificial liver and the extracorporeal liver assist device

Bioartificial liver supporting systems using cryopreserved xenogenic or human hepatocytes have been validated in one large, prospective controlled trial for acute liver failure and primary non-function after liver transplantation. Results are promising as the application is safe, but survival only significantly improved for patients with acute liver failure[150]. Again, data on the application of these bioartificial liver supporting systems for the treatment of PLF in man are lacking.

Rescue hepatectomy and liver transplantation

The use of a rescue hepatectomy followed by liver transplantation (LTx) is based on the concept that the necrotic, failing liver is the source of unknown humoral substances that contribute to the systemic inflammatory response syndrome in patients with liver

failure[151]. LTx is controversial in patients suffering from liver failure after partial hepatectomy for malignant disease and only two trials describe outcomes of LTx for PLF[152, 153]. Otsuka and colleagues showed that LTx was associated with considerable morbidity, but mean survival time was prolonged from 1.4 to 42.2 months. All patients (n = 4) that suffered from PLF but were not appropriate candidates for LTx died, while those undergoing orthotopic LTx survived (n = 7)[153]. However, no criteria are available for the selection of patients that will benefit from emergency LTx for PLF.

ECONOMIC IMPACT

Due to the complex nature of the disease, patients with PLF will need intensive treatment. A study performed in a tertiary referral centre in Germany in 2009 showed that there was a significant difference between the hospital costs of patients with regular recovery after liver surgery (Euro 25,980) versus patients suffering from PLF (Euro 82,199; p=0.013), with a shift of costs towards intensive care[154]. The group of patients suffering from PLF represented 17 per cent of the population, but was responsible for 39 per cent of total hospital costs. These numbers show that PLF is not only a significant clinical, but also economic problem.

RATIONALE OF THE CURRENT THESIS

In the general introduction, the current state of knowledge on liver failure after partial hepatic resection is presented. Although the incidence rate of PLF is relatively low, it is one of the most severe complications of liver surgery and it is the single most important cause of postoperative death. Preventing PLF or improving the outcome of patients suffering from PLF is therefore important in offering potentially curative treatment to patients with liver tumours. Obviously, information on ways to improve outcomes of patients suffering from liver failure after partial hepatectomy would best be derived from high level of evidence research such as RCTs. Unfortunately, such evidence does hitherto not exist and RCTs using PLF as primary endpoint are difficult to perform. The latter is due to the low incidence of PLF, which makes it practically impossible to conduct adequately powered trials using a single component, clinical endpoint as primary endpoint. We therefore investigated ways to facilitate the conduct of high level of evidence research on improving outcomes of patients undergoing liver surgery by using broadly accepted composite and surrogate endpoints.

AIMS OF THE CURRENT THESIS

The broader aim of this thesis was to assess and improve outcomes of patients undergoing liver surgery. This then translated into three more specific aims looking at (1) the feasibility of RCTs in liver surgery using clinical endpoints, (2) surgery-related risk factors of PLF and (3) patient-related risk factors of PLF. Taken together, the aims of the present thesis were as follows:

Aim 1: to develop strategies that increase the feasibility of conducting RCTs in liver surgery using clinical outcomes as primary endpoints (Chapter 2, 3 and 4).

Aim 2: to evaluate the effect of various surgical techniques on hepatic damage in patients undergoing liver surgery (Chapter 5 and 6).

Aim 3: to study the effect of oxaliplatin-based chemotherapy on hepatic damage in patients undergoing partial liver resection for CLMs (Chapter 7, 8, 9 and 10).

OUTLINE OF THE CURRENT THESIS

The present thesis consists of four major parts. In the first part, reasons for the shortage of RCTs in liver surgery are explored and solutions are being offered. Chapter 2 examines the feasibility of RCTs in liver surgery using surgery-related mortality or morbidity as primary endpoint. In chapter 3, standard definitions of liver surgery-related outcomes to be used as clinical endpoints of RCTs in liver surgery are proposed. Chapter 4 describes the development of a liver surgery specific composite endpoint, which can be used as a primary endpoint of future RCTs.

The second part of this thesis focuses on the effect of two common surgical techniques on liver cell damage in patients undergoing liver surgery. Chapter 5 deals with the association between mobilization of the liver and hepatocellular damage and hepatic inflammation in humans. In chapter 6, an RCT analyzing the effect of 15 or 30 min intermittent Pringle maneuver on liver cell damage during liver surgery in humans is described.

Part III focuses on hepatic damage secondary to oxaliplatin-based chemotherapy in patients with CLMs. Chapter 7 illustrates the clinical consequence of hepatic sinusoidal injury in patients treated with oxaliplatin prior to liver surgery for CLMs. Chapter 8 explores the value of systemic hyaluronic acid level as preoperative, non-invasive marker of SOS. The impact of sinusoidal injury of the non-tumour-bearing liver on tumour regression grade is examined in chapter 9. Chapter 10 analyzes the preventive effect of the flavonoid monoHER on hepatocellular and sinusoidal injury in an experimental rat model.

The fourth part of this thesis encompasses a summary and discussion of the findings described in the first three parts (chapter 11 and 12). In chapter 13 and 14, clinical implications and directions for the future are emphasized.

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Part I

Assessing outcomes of liver surgery: critical appraisal of clinical endpoints

Chapter 2

Feasibility of randomized controlled trials in liver surgery using surgery-related mortality or morbidity as endpoint

Published as:

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ABSTRACT

Background

There is a shortage of randomized controlled trials (RCTs) on which to base guidelines in liver surgery. The feasibility of conducting an adequately powered RCT in liver surgery using the dichotomous endpoints surgery-related mortality or morbidity was studied.

Methods

Articles published between January 2002 and November 2007 with mortality or morbidity after liver surgery as primary endpoint were retrieved, based on which mean incidence rates were calculated. Sample size calculations for an RCT aiming to show a relative reduction of these endpoints by 33, 50 or 66 per cent were performed.

Results

The mean operative mortality rate was 1.0 per cent and the total morbidity rate 28.9 per cent; mean rates of bile leakage and post-resectional liver failure were 4.4 and 2.6 per cent, respectively. The smallest numbers of patients needed in each arm of an RCT aiming to show a one-third relative reduction were 15 614 for operative mortality, 412 for total morbidity, 3 446 for bile leakage and 5 924 for post-resectional liver failure.

Conclusion

The feasibility of conducting an adequately powered RCT in liver surgery using dichotomous outcomes such as mortality or specific complications seems low. Conclusions of underpowered RCTs should be interpreted with caution. Implementation of a liver surgery specific composite endpoint may be a useful and clinically relevant solution to pursue.

INTRODUCTION

The application of evidence-based medicine aims to improve rational and consistent decision making. It combines the best available external clinical evidence with individual clinical expertise[1]. Evidence-based guidelines should preferably be based on the results of randomized controlled trials (RCTs) that test the effectiveness of a new treatment against the standard treatment with respect to a predetermined, clinically relevant endpoint. However, RCTs are scarce in liver surgery[2].

The safety of partial liver resection has improved considerably in the past decade[3-5]. As curative oncological resections are being performed for even larger tumours, the minimum remnant liver volume is constantly being re-evaluated. As a result, complications such as bile leakage, intra-abdominal abscess or post-resectional liver failure remain a major threat to the patient. RCTs testing the efficacy of innovative techniques in lowering these complication rates are desirable.

RCTs that use dichotomous endpoints such as surgery-related mortality, total morbidity or specific complications as primary endpoints generate the strongest evidence and are necessary to obtain approval of the US Food and Drug Administration. However, these endpoints might increase the complexity of an RCT because they often necessitate large sample sizes. A state-of-the-art calculation of the smallest number of patients needed to be enrolled is an essential first step in the design of a randomized trial. If too few patients are enrolled, a trial testing a treatment with a clinically important treatment effect may fail to show a statistically significant effect owing to lack of statistical power, whereas inclusion of too many patients may be regarded as unethical, costly and inefficient. Unfortunately, the performance and reporting of sample size calculations is suboptimal in both the surgical and hepatobiliary literature[2, 6, 7].

The aim of the present study was to determine the feasibility of conducting an adequately powered RCT in liver surgery using the dichotomous endpoints surgery-related mortality or morbidity. A systematic review was undertaken to estimate the current rates of mortality, total morbidity and specific complications of liver surgery. Based on these rates, the minimum sample size needed to conduct an adequately powered RCT that would be able to show a relative reduction in surgery-related mortality or morbidity by at least one-third was calculated.

METHODS

Search strategy

A systematic, computerized literature search (PubMed, the Cochrane Library and Embase) was performed using the key words "hepatectomy OR liver resection" in order

to identify all studies that described clinical outcomes of liver surgery. The search was conducted to determine the rates of liver surgery-related mortality, total morbidity and specific complications in current HPB practice. The search term was limited to articles published in English between January 2002 and November 2007 that included patients older than 19 years of age. Article reference lists were cross-checked for additional useful papers to increase the sensitivity of the search. The outcome measures were 30-day, operative and/or in-hospital mortality and total morbidity, procedure specific morbidity (bile leakage and post-resectional liver failure) and other types of morbidity, depending on the outcome measures reported.

Study eligibility

Studies were eligible if they were consecutive case series, came from high-volume centres (carrying out more than 15 resections per year[8]), included a minimum of 50 patients, and reported on a case mix of patients and resections. Patients undergoing repeat resections were also included. Each article had to describe the study interval, study characteristics (patient number and presence of exclusion criteria), operative data (total number of resections), one mortality index (30-day, operative or in-hospital mortality), total morbidity and at least one procedure specific complication. Procedure specific complications were defined as bile leakage and post-resectional liver failure. When the same institute reported two studies using data from overlapping periods, the one of better quality was selected based on a combination of the criteria reported by Martin and colleagues and Moher and co-workers[9, 10]. The methodological quality of these papers was assessed by a sum score. An RCT scored 5 points, a prospective trial 3 points, and all other positive quality items on the checklist scored 1 point, leading to a maximum quality score of 19 points (Appendix 1).

Studies were excluded if they (1) reported on other outcomes or were irrelevant to the subject under study (for example animal studies or studies on liver transplantation), (2) used aggregated data from nation-wide data sets, (3) only described a specific patient group, (4) were reviews, (5) lacked detailed data on either mortality or total or procedure specific morbidity.

Selection and data extraction

Two authors independently performed the computerized search and selected abstracts that met the predetermined eligibility terms for full-text examination. Thereafter, two authors judged the suitability of these studies for inclusion in the final analysis by using a standardized checklist (Appendix 1, items marked with an asterisk). Data of the selected studies were extracted by one author and independently reviewed by two other authors. Disagreements were resolved by discussion.

The following data were recorded systematically: first author, year of publication, institute name and location, continent of origin, study design, study interval, number of patients, sex, age, number, type and indication for liver resection, presence of data on and definition of procedure specific complications (bile leakage and post-resectional liver failure), and duration of follow-up. The incidence of the following post-resectional complications was recorded, if explicitly mentioned: 30-day, operative or in-hospital mortality, total morbidity, post-resectional liver failure, bile leakage, ascites, sepsis, intra-abdominal abscess, ileus, pleural effusion, pneumonia, pneumothorax, urinary tract infection, renal insufficiency, cardiac arrhythmia, intra-abdominal hemorrhage, wound infection and dehiscence.

Definition of outcome measures

Resections were regarded as major when three or more segments were involved and minor when one or two segments were involved or a non-anatomical resection was performed[11].

Mortality was classified into 30-day, operative and in-hospital mortality, dependent on the mortality index used in the article. Total morbidity included all complications that prolonged the patients' hospital stay or had a life-threatening impact, dependent on the post-resectional complications accounting for total morbidity per article. Self-limiting post-operative events were regarded as minor complications, and were grouped as grade 1 or 2 according to the classification index of Dindo and colleagues[12]. Major complications comprised those requiring surgical, endoscopic or radiological intervention, or resulting in multi-organ failure or death (Dindo grades 3 to 5).

Mortality and morbidity rates after liver resection

The rates of surgery-related mortality, total morbidity and specific complications in current HPB practice were calculated by means of a mixed effects logistic regression meta-analysis. Mean rates and between-study variance (heterogeneity) of the rates were determined for each complication. Only complications that were documented by at least half of the studies selected for final analysis were included in the mixed effects logistic regression meta-analysis. For surgery-related mortality and total morbidity, these meta-analyses were repeated with design (prospective versus retrospective) as predictor of the mean incidence rate.

Sample size calculation

Sample size was calculated based on these mean rates with the assumption that an (hypothetical) RCT should be able to detect a clinically meaningful, relative reduction in surgery-related mortality, total morbidity or specific complications by one-third, one-half or two-thirds. The null-hypothesis was that there was no difference between the

intervention and standard treatment in the incidence rate of the primary endpoint. All sample size calculations assumed two-sided testing.

The sample size of each arm was calculated using the equation designed for two proportions (as described by Kirkwood and colleagues[13] and illustrated in Appendix 2); α was set at 0.05 to control for type I error (false-positive result) and β at 0.10 to control for type II error (false-negative result). Only one primary endpoint was chosen for each hypothetical RCT, as testing for multiple outcome parameters would require correction for multiple comparisons and thereby increase the sample size.

Effect of change in study design on sample size

The calculated sample sizes were based on a single-centre trial with no dropouts and a level of significance α = 0.05 and power 1-β = 0.90. Changes in these assumptions result in changes in the sample size.

Random dropout

The expected loss to follow-up owing to random dropout from the intervention and control groups was taken into account by increasing the sample size with the correction factor = 100 / (100-K) where K represents the percentage dropout. This is a remedy against loss of power owing to random dropout only, and is not a solution to bias because of selective dropout, which requires advanced statistics[14].

Multicentre trial

A larger patient population can be obtained by running a multicentre trial. However, centre and treatment-by-centre effects on the outcome increase the sample size needed for a given effect size and power. Without treatment-by-centre interaction, an increase of the sample size of each arm of an RCT with C, where C denotes the number of centres, is sufficient. With interaction, however, a larger sample size is needed and its calculation depends on whether centres are treated as fixed or random, and on whether the feasible sample size varies strongly between centres[15, 16].

Change in A and B terms

A and B terms (α and β respectively) are set at the beginning of a study, but their values are open to discussion. An α of 0.01 instead of 0.05 may be considered in case of multiple outcomes (Bonferroni correction for multiple comparisons). A β of 0.20 instead of 0.10 implies a power of 80 per cent, which is often used in planning studies.

Statistical analysis

Statistical analysis of study characteristics was performed using SPSS version 12.0.1 for Windows (SPSS Inc., Chicago, IL, USA). The mixed effects logistic regression meta-analysis

was performed using second order penalized quasi-likelihood estimation with MLwiN 1.100007 (Centre for Multilevel Modelling, Bristol, UK)[17]. This resulted in a mean incidence rate with 95 per cent interval for individual studies for each complication. Essentially, the μ and σ^2 as estimated by mixed effects logistic regression are the between-study mean and variance of the incidence on the logodds scale. Mean on the percentage scale was obtained by taking μ and then applying the inverse logodds transformation. The 95 per cent interval was obtained by taking $\mu \pm 2\sigma$ and then applying the inverse logodds transformation to the interval boundaries multiplying by 100 per cent. Heterogeneity (between-study variance) was tested using a chi-square test of σ^2 (ratio of $[\sigma^2 / SE]^2$) with a cutoff value of more than 4 (two-sided testing and $\alpha = 0.05$). Sample sizes are presented as absolute numbers and represent the minimum number of patients needed in each arm of an RCT.

RESULTS

Search strategy

A total of 2 666 abstracts that met the search terms "hepatectomy OR liver resection" were retrieved. Of these, 2 549 were excluded after abstract review (Figure 2.1). The main reasons for exclusion were irrelevance to the subject under study (*n* = 926), small patient numbers (*n* = 519 case reports and *n* = 351 studies with fewer than 50 patients) and reporting on other outcomes (*n* = 314). Some 117 potentially eligible articles underwent full text examination. Nineteen of these contained overlapping data, and the ten articles of inferior quality were excluded (Appendix 3). Additional reasons for exclusion are listed in Figure 2.1. Hand searching of the reference lists identified two additional eligible articles. Finally, 32 studies fulfilled all inclusion criteria and were used in the analysis (Appendix 4)[3, 18-48].

Study and patient characteristics

The 32 studies contained a total of 8 680 patients who underwent 8 824 resections (Table 2.1). The median number of patients per study was 128 (range 53 – 1 803). Twenty studies were conducted prospectively, nine of which were RCTs; ten were retrospective and two lacked this information. The methodological quality of these 32 articles is summarized in Appendix 4. Fifteen articles reported data originating from European centres, fourteen from Asian centres and three from other continents (one each from North-America, South-America and Australasia).

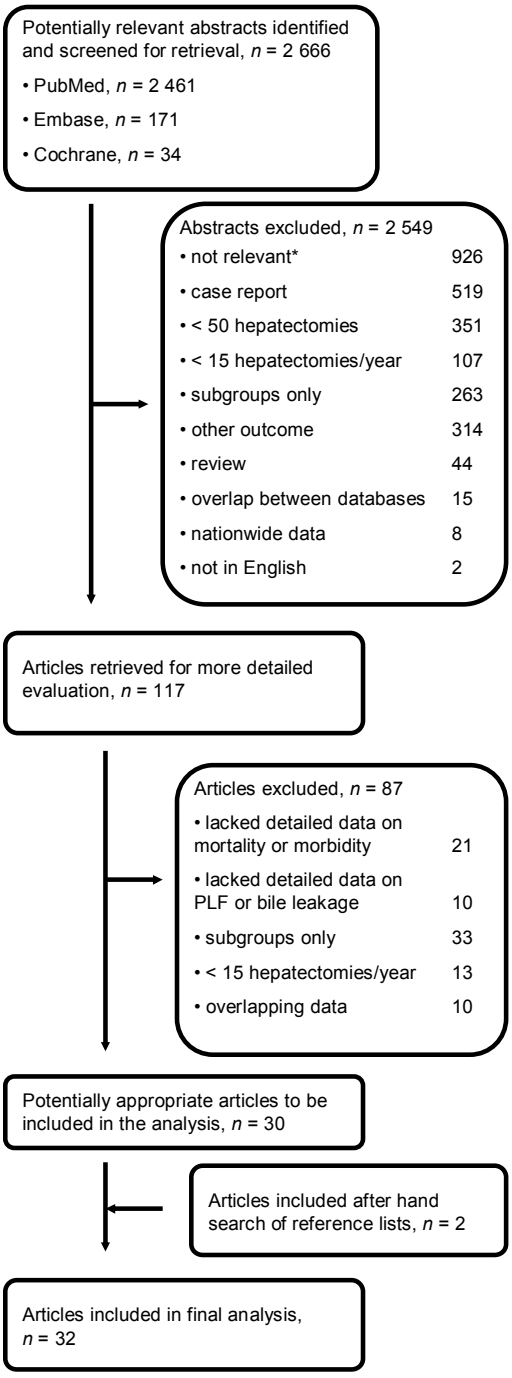

Figure 2.1 Selection of studies eligible for final analysis.
Legend: PLF, post-resectional liver failure; Hx, hepatectomy; * e.g. animal studies, genetic studies, studies focussing on liver transplantation.

Table 2.1 Characteristics of patients described in the articles eligible for final analysis.

Characteristics	***N* = 8 680**
Sex ratio (female : male)	2 : 3
Age (years) (range)*	0.2 – 89.0
Indication for resection†	
Primary hepatic malignancy	3 948 (46.6)
Secondary hepatic malignancy	3 484 (41.1)
Benign hepatic disease	739 (8.7)
Other	307 (3.6)
Number of resections	8 824
Type of resection‡	
Major hepatectomy	3 577 (47.5)
Minor hepatectomy	3 960 (52.5)

Numbers in parentheses are percentages; * four studies included liver resections in children[29, 37, 43, 45]; † data missing from three studies[20, 38, 43]; ‡ data from three studies not compatible[20, 25, 39].

Mortality and morbidity rates after liver resection

The distribution of rates of surgery-related mortality and morbidity is shown in Figure 2.2 (and Appendix 4). Twenty-nine articles reported the incidence of bile leakage and 25 articles the rate of post-resectional liver failure; 22 reported both. Nine articles provided

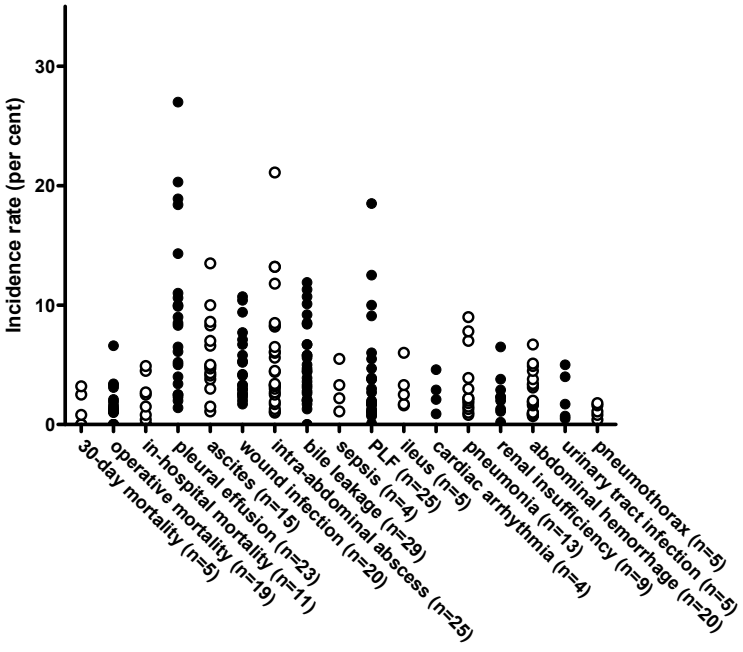

Figure 2.2 Distribution of rates of liver surgery-related mortality and specific complications.
Legend: *N*, number of articles reporting on the complication; PLF, post-resectional liver failure.

a definition of bile leakage and 10 of post-resectional liver failure. Definitions used for these procedure specific complications varied widely. Complications were reviewed during the in-hospital stay in four studies[21, 34, 40, 45], and during the first 30, 180 and 360 days after operation in three[23, 24, 41], one[22] and one[27], respectively. This information was lacking in the other 23 articles. Complications were described according to the Clavien-Dindo classification in four articles only[19, 23, 24, 47].

The mean rates of surgery-related mortality and specific complications reported by at least 16 articles were low (Table 2.2). Mean operative mortality rate was 1.0 per cent and total morbidity rate 28.9 per cent. Rates were 7.2 per cent for pleural effusion, 4.6 per cent for intra-abdominal abscess and wound infection, 4.4 per cent for bile leakage, 2.6 per cent for post-resectional liver failure and 1.9 per cent for intra-abdominal hemorrhage. The between-study variance (heterogeneity), depicted as σ^2 and 95 per cent intervals in Table 2.2, was significant for all complications except operative mortality and intra-abdominal hemorrhage (which may be due to the small number of studies reporting on these complications).

Mean incidence rates of operative mortality and total morbidity differed between studies with a prospective or retrospective design (1.1 versus 0.7 per cent for operative mortality and 27.1 versus 33.9 per cent for total morbidity). However, these differences were non-significant (p=0.562 and p=0.153, respectively).

Table 2.2 Mean rates of surgery-related mortality, total morbidity and specific complications after liver resection.

Complication	*N*	μ logodds*	σ^2 logodds*	mean rate†	95 per cent interval‡
Operative mortality	19	−4.558 (0.316)	1.050 (0.576)	1.0	0.1 – 7.5
Total morbidity	30#	−0.902 (0.105)	0.288 (0.085)	28.9	12.2 – 51.9
Specific types of morbidity					
1. Pleural effusion	23	−2.551 (0.184)	0.632 (0.225)	7.2	1.6 – 27.7
2. Wound infection and dehiscence	20	−3.032 (0.141)	0.242 (0.121)	4.6	1.8 – 11.4
3. Intra-abdominal abscess	25	−3.028 (0.173)	0.569 (0.207)	4.6	1.1 – 18.0
4. Bile leakage	29	−3.089 (0.139)	0.386 (0.142)	4.4	1.3 – 13.6
5. Post-resectional liver failure	25	−3.635 (0.247)	1.152 (0.419)	2.6	0.3 – 18.4
6. Intra-abdominal hemorrhage	20	−3.951 (0.181)	0.328 (0.193)	1.9	0.6 – 5.7

N, number of articles reporting on the complication; * numbers in parentheses are standard error; † computed by inverse logodds transform of μ, giving a proportion, and multiplying by 100 per cent; ‡ computed by inverse logodds transform of the interval boundaries $\mu \pm 2\sigma$ and multiplying by 100 per cent; # data on total morbidity, reported in two studies[24, 47], were divided into major and minor morbidity and were therefore not compatible.

Sample size calculation

The low rates of surgery-related mortality and morbidity would necessitate a large number of patients in each arm of an adequately powered RCT that aimed to show a relative reduction of the incidence of a complication by one-third, one-half or two-thirds (Table 2.3). For example, if the intention was to show a one-third reduction in the incidence of bile leakage, that is a reduction from the reported 4.4 to 2.9 per cent, 3 446 patients would be needed in each arm of a trial to have sufficient power. For an RCT aiming to show a relative reduction in bile leakage by one-half or two-thirds, 1 383 or 693 patients, respectively, would be required in each arm.

Because of its much higher rate, use of total morbidity as endpoint would considerably decrease the number of patients needed in each arm of an adequately powered RCT intending to show a reduction by one-third, one-half or two-thirds (Table 2.3). However, total morbidity was ill-defined in most articles.

Table 2.3 Sample size required for each arm of a randomized controlled trial intending to show a relative reduction of surgery-related mortality, total morbidity or specific complications by one-third, one-half or two-thirds.

	Relative reduction		
Complication	**33%**	**50%**	**66%**
Operative mortality	15 614	6 250	3 127
Total morbidity	412	169	86
1. Pleural effusion	2 054	826	415
2. Wound infection and dehiscence	3 290	1 320	662
3. Intra-abdominal abscess	3 290	1 320	662
4. Bile leakage	3 446	1 383	693
5. Post-resectional liver failure	5 924	2 374	1 189
6. Intra-abdominal hemorrhage	8 155	3 266	1 635

Assumptions $\alpha = 0.05$ and $\beta = 0.1$ and two-sided testing.

Effect of change in study design on sample size

To take into account a random dropout of 10 per cent, the sample size of each arm of an RCT should be increased by 11 per cent. The effects of conducting a multicentre study were less dramatic, at least when assuming an absence of treatment-by-centre interaction. A study with 20 centres would need to include only an extra 20 patients in each arm.

The effects of changes in A and B terms on the sample size of an RCT aiming to show a relative reduction of bile leakage by 33, 50 or 66 per cent are illustrated in Figure 2.3. A change in power from 90 per cent ($\beta = 0.1$) to 80 per cent ($\beta = 0.2$) resulted in a decrease of the sample size by approximately 25 per cent (dotted line in Figure 2.3). Changing α from 0.05 to the more stringent 0.01 increased the sample size by 42 per cent (striped line in Figure 2.3).

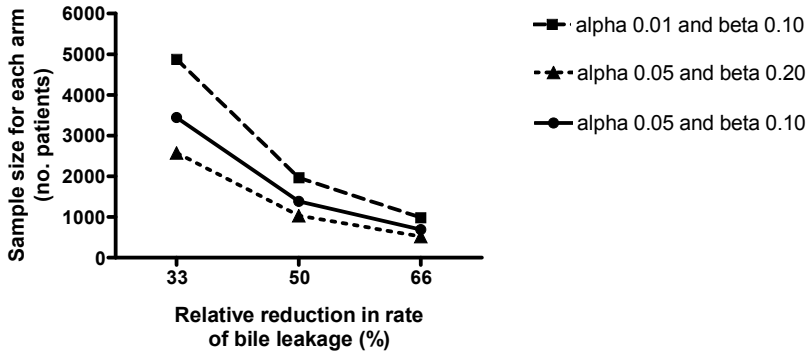

Figure 2.3 The effect of change of α or β on the sample size of each arm of a randomized controlled trial that intends to show a relative reduction of bile leakage by one-third, one-half or two-thirds.

DISCUSSION

The current rates of surgery-related mortality and specific types of morbidity after liver resection were low. Operative mortality rate was only 1.0 per cent and the rates of specific types of morbidity were all below 8 per cent. Consequently, the sample size of an adequately powered RCT using surgery-related mortality or specific types of morbidity as primary endpoint turned out to be extremely large. The smallest numbers of patients to be included in each arm of an RCT that aims to show a relative reduction by one-third were 15 614 for operative mortality, 412 for total morbidity, 3 446 for bile leakage and 5 924 for post-resectional liver failure. Studies using fewer patients can be regarded as underpowered and their results should be interpreted with caution.

This study is limited to short-term surgery-related outcomes. A similar estimation for long-term outcomes after liver surgery requires a different search strategy and was beyond the scope of the present study. Rates of surgery-related mortality, total morbidity and specific types of morbidity after liver resection are gradually decreasing due to better preoperative work-up, operative strategies and perioperative patient care[24, 32, 49]. The present analysis shows that, because of these declining event rates, it will be increasingly difficult to conduct an adequately powered RCT using a clinical endpoint as primary endpoint. Our Liver Unit is a high-volume centre for liver surgery in the Netherlands and performs approximately 70 liver resections annually. Conduction of a single-centre RCT in our Unit with the intention to show a relative reduction of bile leakage by one-third would require an inclusion period of 99 years. Even in the largest Liver Units in the world (performing about 200 hepatectomies annually[3, 40, 45]), this RCT would take 35 years. Inclusion periods of these lengths are unworkable; moreover, by the end of the trial, any conclusions would no longer be relevant. In addition, the feasibility of a multicentre trial is questionable because only 500 liver resections are performed annually in the Neth-

erlands and only 7 000 in the USA (Netherlands Health Care Inspectorate 2005, www.prismant.nl, and HCUP Nationwide Inpatient Sample 2000, www.hcup-us.ahrq.gov).

Underpowered RCTs are ineffective because the accuracy of their results is uncertain. They might lead to the generation of potentially harmful conclusions that are incorporated in treatment guidelines. The CONSORT statement was developed to overcome this problem[10]. It contains 22 criteria on study design and sample size calculations for RCTs. Although the quality of reported trials improved after the introduction of the CONSORT statement[50], overall reporting on RCTs in surgical journals is still suboptimal[7, 9].

The minimum number of patients to be included in an RCT might have been overestimated in the present study. Possible confounders are the inclusion of articles of suboptimal quality (for example those lacking definitions of procedure specific complications, those with short follow-up periods, or retrospective case series[9, 51]), publication bias resulting from selective publication of results from centres of excellence, or the inclusion of studies that assessed a case mix of patients and resections. Although the latter leads to an increased external validity, it may not reflect the actual complication rate in a subgroup of patients.

The overall quality of the selected articles was suboptimal with reporting of both procedure specific complications in only two-thirds and these complications defined in less than half. Furthermore, data collection was retrospective in one-third of studies, and such studies have been shown to be of inferior quality compared with prospective case series[9]. However, no significant differences in rates of operative mortality and total morbidity were found in the present meta-analysis between prospective and retrospective studies.

The feasibility of an RCT could be enhanced by including selective patient groups (such as patients undergoing major liver resection or patients with cirrhosis) or by choosing composite or surrogate endpoints. Some studies have identified major liver resection as an independent predictor of post-resectional morbidity[27, 45]. Patients who have undergone major liver resection have higher complication rates and, consequently, smaller sample sizes will be needed. On the other hand, the number of eligible patients will also decrease.

Total morbidity, probably the only outcome resulting in a feasible sample size, was ill-defined and therefore not ideal as a primary endpoint of an RCT. An alternative would be to use a well-defined liver surgery specific composite endpoint, that is a parameter composed of a combination of two or more procedure specific complications that are considered as a single, dichotomous outcome. This increases the event rate and prevents multiplicity, both of which increase the statistical power of an RCT. The authors propose a composite endpoint comprising operative mortality, bile leakage, intra-abdominal abscess, post-resectional liver failure, and intra-abdominal hemorrhage. Such an endpoint is dichotomous and reflects the occurrence of at least one of these components,

which are all specific to liver surgery and have substantial clinical relevance, reflecting a Clavien-Dindo severity grade of 3 or more[12]. Based on the rates per component (Table 2.2), the incidence of the liver surgery specific composite endpoint can be estimated to lie between 4.6 and 14.5 per cent, depending on the presence of simultaneous complications (the occurrence of two or more of these endpoints in one patient), which is undesirable. A prospective study is necessary to determine the true incidence and validity of this composite endpoint as the individual components might have variable clinical relevance or rates, dependent on the patient cohort under study[52].

Surrogate endpoints are increasingly being used as they reduce the numbers of patients needed in RCTs by transforming the primary endpoint from a dichotomous into a continuous variable. An essential characteristic of a surrogate endpoint is the ability to reflect the clinical benefit of an intervention accurately. Surrogate endpoints that have been used recently to describe post-resectional outcomes are rather weak, including the biomarkers serum bilirubin and prothrombin index (combined in the so-called 50-50 criteria) for post-resectional liver failure[53], rise in serum aminotransferase level for ischemia-reperfusion injury[49] and plasma arterial lactate for mortality[21]. Functional parameters used as surrogate endpoints consist of time to passage of flatus for ileus[24] or length of hospital stay for total morbidity. However, there is currently no consensus about the validity of these surrogate endpoints, which is a prerequisite for approval of new drugs by the US Food and Drug Administration. Future research should focus on the validation of convincing surrogate endpoints in large prospectively followed cohorts.

The mandatory institution of prospective registries to monitor the safety and therapeutic effectiveness of innovative techniques in HPB surgery that have not been tested by adequately powered RCTs, as suggested by Strasberg and Ludbrook[54], seems also interesting. These registries could overcome the problem of lack of statistical power of RCTs by compiling high-volume data on mortality or complications after new procedures. The occurrence of unexpected adverse events following the introduction of a procedure could be identified by such a registry despite their low incidence; an example is the increased rate of biliary injury after the introduction of laparoscopic cholecystectomy[54].

In summary, to achieve the required sample size to conduct an adequately powered RCT in liver surgery using the dichotomous endpoints mortality, bile leakage or post-resectional liver failure, multicentre collaborations or protracted inclusion periods would be required. However, multicentre trials of this size do not seem realistic. Implementation and validation of a liver surgery specific composite endpoint might increase the feasibility of conducting RCTs in liver surgery.

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Chapter 3

Proposal for uniform definitions of clinical outcomes of liver surgery: results of a web-based survey

Adapted from:

van den Broek MA, van Dam RM, van Breukelen GJ, Bemelmans MH, Oussoultzoglou E, Pessaux P, Dejong CH, Freemantle N, Olde Damink SW. Development of a composite endpoint for randomized controlled trials in liver surgery. *Br J Surg* 2011; 98: 1138-45.

ABSTRACT

Background

In liver surgery, high level of evidence research, such as randomized controlled trials (RCTs) using clinical outcomes as primary endpoint, is desirable but difficult to perform. Complication rates are so low that evaluation of clinical outcomes results in large sample sizes. The use of composite endpoints and conduct of meta-analyses may enable the performance of high level of evidence research in liver surgery. Their implementation is hampered by the lack of uniform definitions of complications of liver surgery. The present study aimed to develop standard definitions of liver surgery-related clinical outcomes.

Methods

A systematic literature search was performed to identify clinical endpoints of contemporary RCTs in liver surgery. A web-based survey was composed and sent to 54 international experts in HPB surgery in order to reach consensus on definitions of the most frequently used clinical outcomes. During 2 consensus meetings, the proposed definitions were adapted by the study group according to suggestions of the respondents.

Results

The literature search yielded 47 RCTs that used clinical outcomes as primary or secondary endpoint. Clear definitions of these clinical outcomes were provided in less than one-third of trial reports and varied widely. The 12 most commonly used clinical endpoints included (in descending order): operative mortality, bile leakage, blood transfusion, intra-abdominal abscess, wound infection, intra-abdominal hemorrhage, post-resectional liver failure, pneumonia, ascites, pleural effusion, sepsis, and acute renal failure. These endpoints formed the basis of the web-based survey. Thirty-one responses were collected (response rate of 57 per cent). Based on the comments of the respondents, uniform definitions of aforementioned clinical outcomes were proposed.

Conclusion

Consensus-based, standard definitions of frequently used, clinical outcomes of liver surgery are proposed. These definitions can be used to uniformly describe clinical endpoints of RCTs in liver surgery, which will facilitate the conduct of meta-analyses in the future. Furthermore, they can be used to define components of a liver surgery specific composite endpoint.

INTRODUCTION

The decision to adopt new surgical techniques is ideally based on results of adequately powered, randomized controlled trials (RCTs) using meaningful endpoints. These endpoints may refer to clinical, efficacy or patient-reported outcomes. This evidence-based approach of surgical innovation calls for proper trial design[1, 2].

A critical step in the design of a trial is the selection of an appropriate primary endpoint. The optimal primary endpoint allows for unambiguous, efficient and objective evaluation of the study intervention[3]. In liver surgery, single component, clinical outcomes such as surgery-related mortality or specific complications are relevant primary endpoints. However, they are of limited use as their event rate is so low that evaluation results in unfeasibly large sample sizes and long inclusion periods[4, 5]. In response, trialists can choose for the performance of meta-analyses or use of composite endpoints (CEPs) as primary endpoints.

The success of performing meta-analyses and implementing CEPs in the field of HPB surgery is, among other factors, dependent on the presence of pre-specified definitions of clinical outcomes of liver surgery[6-8]. Up until now, uniform definitions of many complications of liver surgery are lacking. As an example, definitions of the liver surgery specific complications bile leakage and post-resectional liver failure varied widely, which resulted in highly variable reported incidence rates[5, 8, 9]. In other medical disciplines, joint efforts already led to consensus-based proposals for standard definitions (for example [10, 11]). The use of standard definitions reduces inconsistencies in trial reporting, allows for unequivocal interpretation of trial data and facilitates comparison of trial results[8, 10, 12].

The present study was designed to develop uniform definitions of clinical outcomes of liver surgery.

METHODS

A multi-step approach was applied to obtain consensus on definitions of clinical outcomes of liver surgery. A structured literature search was conducted to identify clinical endpoints used in contemporary RCTs in liver surgery. Definitions of these clinical endpoints were extracted from the trial reports. Based on these results, a web-based survey was designed, which consisted of two parts. The first part included proposals for definitions of clinical outcomes of liver surgery. The second part focussed on the selection of components of a liver surgery specific CEP and is described in chapter 4 of the present thesis.

A. Systematic literature search to identify clinical endpoints of RCTs in liver surgery

Search strategy and study eligibility

An electronic search was performed to identify all single component, clinical outcomes used as primary or secondary endpoints of RCTs in liver surgery. PubMed, the Cochrane Library and Embase were searched using the terms "liver resection OR hepatic resection OR hepatectomy" combined with "randomized controlled trial". Related terms were also included. The search was limited to articles that included patients older than 19 years of age and were published in English between January 2004 and December 2008. Reference lists were cross-checked for additionally eligible papers.

Studies were eligible if they (1) were RCTs, (2) reported on outcomes of liver surgery, (3) reported on human subjects, and (4) used dichotomous, clinical outcomes as either primary or secondary endpoints. Studies were excluded if they (1) reported on non liver surgery-related outcomes (were not relevant), (2) used surrogate outcomes only as primary and secondary endpoints, (3) described a study protocol of an RCT, (4) were published in abstract form only or (5) could not be retrieved.

Selection and data extraction

Abstracts of RCTs were screened by one author. Full-texts of potentially eligible RCTs were retrieved via the internet or from the corresponding author. A standard checklist was used for full-text examination, performed by two authors who decided on inclusion for final analysis. Disagreements were resolved by discussion. Data on study origin, design, sample size, study interval, type of intervention, primary and secondary endpoints, and study outcomes as well as definitions of clinical endpoints were recorded systematically. If the primary endpoint was not mentioned explicitly, the endpoint used for sample size calculations was considered the primary endpoint.

B. Web-based survey on definitions of complications of liver surgery

The clinical endpoints most frequently used in the selected RCTs formed the basis of a web-based survey. The survey was designed using commercially available survey software (www.surveymonkey.com) and consisted of two parts. The intention of this survey was (1) to reach consensus on definitions of complications of liver surgery (part I) and (2) to select components of a future liver surgery specific CEP (part II, described in chapter 4 of the present thesis). Directors of HPB units worldwide were invited to participate in the survey. The survey opened in February 2009 and closed in July 2009. Reminder e-mails were sent after 1 and 3 months and, if necessary, directors were personally contacted.

This paper deals with the results of part I of the survey. Part I consisted of questions on definitions of complications of liver surgery and their severity grade. The presented definitions were derived from the selected RCTs and other key papers in the field. Attributes

of the proposed definitions were that they were (1) consistent, (2) self-explanatory, (3) linguistically correct, (4) precise, (5) concise, (6) time-dependent, and (7) objective (adapted from [13]). The cutoff for approval of the definition was set at more than two-thirds of respondents indicating that they agreed with the proposed definition. Each proposed definition was supplemented with additional questions on complication-related topics and a comment box was available for replies.

Initially, severity grading was performed using a grading system designed by Ferreiro-Gonzales and colleagues[14]. After analysis of the first ten replies, this severity grading system was abandoned as it was considered too subjective. The more objective Clavien-Dindo severity grading system, which is based on the intervention required for a complication, was adopted thereafter[15]. In short, any deviation from the postoperative course without the need for pharmacological, radiological or surgical intervention was classified as Clavien-Dindo grade 1; complications requiring pharmacological treatment were graded as grade 2; complications requiring surgical or radiological intervention not under general anaesthesia were classified as grade 3a and under general anaesthesia as grade 3b; grade 4 complications were life-threatening complications requiring intensive care because of single organ dysfunction (grade 4a) or multi-organ dysfunction (grade 4b); death was classified as grade 5.

C. Consensus on definitions of complications of liver surgery

After collection of all replies, the proposed definitions were adjusted according to comments of the respondents during two meetings of the project group. The project group consisted of the authors of the present paper.

RESULTS

A. Systematic literature search

The electronic search identified 326 abstracts that fulfilled the search terms. Main reasons for exclusion are shown in Figure 3.1. No additional RCTs were identified by hand searching the reference lists. Finally, 47 RCTs were included in the analysis[16-62]. Their summarized characteristics are depicted in Table 3.1 and Appendix 5. Most trials were published by European (n = 26) or Asian (n = 20) centres and focussed on interventions with medication (n = 17) or new surgical techniques (n = 15). Eighteen RCTs had a single component, dichotomous primary endpoint and twenty-nine a surrogate, continuous primary endpoint (Appendix 5). Mean sample size of the included RCTs was 100 patients (range 20 – 364 patients).

Table 3.2 depicts the single component, clinical endpoints that were used as either primary or secondary endpoints and the number of times they were used. A minority of

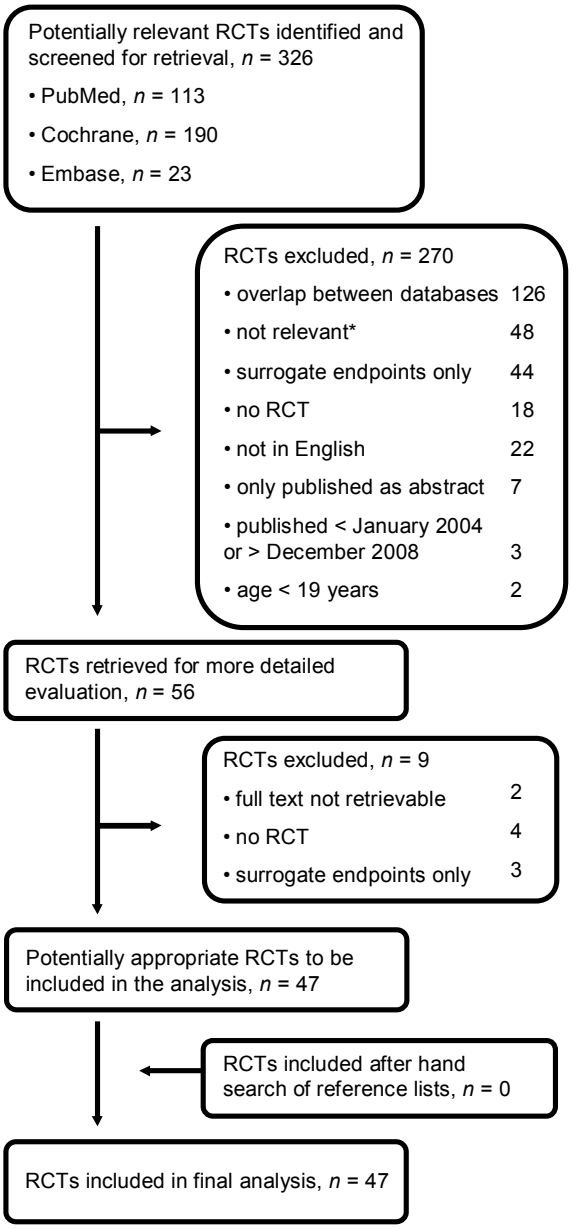

Figure 3.1 Flowchart of study selection.
Legend: RCT, randomized controlled trial; *N*, number; * e.g. RCTs on liver transplantation or pancreatic surgery.

trial reports explicitly stated definitions of these clinical endpoints (Table 3.2), except for the endpoint mortality. A detailed overview of the definitions provided by the selected RCTs can be found in Appendix 6.

Table 3.1 Characteristics of the selected randomized controlled trials.

Characteristics	***N* = 47**
Continent of origin	
Asia	20 (43)
Europe	26 (55)
North America	1 (2)
Type of intervention	
Medication	17 (36)
New surgical technique	15 (32)
Technical device	7 (15)
Other*	8 (17)
Multicentre trial	5 (11)
Clinical, dichotomous endpoint as primary endpoint	18 (38)
Single component endpoint	10
Composite endpoint	8
Surrogate, continuous endpoint as primary endpoint	29 (62)
Mean sample size (patients) (range)	100 (20 – 364)

Numbers between parentheses are percentages unless otherwise indicated; *N*, number; * e.g. type of blood transfusion.

Table 3.2 Single component, clinical outcomes used as primary or secondary endpoints in the selected randomized controlled trials (*n* = 47).

Endpoint	***N* times used as primary or secondary endpoint in the selected RCTs***	***N* times explicitly defined in the selected RCTs****
Operative, 30-day, in-hospital mortality	38	34
Biliary leakage, biliary fistula, bilioma	35	10
Blood transfusion	34	0
Intra-abdominal, perihepatic or subphrenic abscess	28	6
Wound infection, wound dehiscence	27	4
Intra-abdominal hemorrhage, bleeding	25	1
Post-resectional liver failure, hepatic dysfunction	24	11
Pneumonia	23	4
Ascites	21	6
Pleural effusion	20	0
Sepsis, septicemia	9	1
Acute renal failure	7	1
Ileus	6	0
Pulmonary embolus	6	0
Urinary tract infection	6	0
Atelectasis	5	1

Table 3.2 Continued

Endpoint	*N* times used as primary or secon-dary endpoint in the selected RCTs*	*N* times explicitly defined in the selected RCTs**
Myocardial infarction	5	1
Portal vein thrombosis	5	0
Gastro-intestinal perforation	3	0
Gastro-intestinal bleeding	2	0

N, number; RCT, randomized controlled trial; * clinical endpoints used in studies reported by the same institute using data from overlapping time periods were taken into account once (Appendix 5); ** a complete list of definitions is provided in Appendix 6.

B. Results of the web-based survey

The 12 single component, clinical outcomes most frequently used as primary or secondary endpoints in the selected RCTs (Table 3.2) formed the basis of the web-based survey. An example of the survey can be found in Appendix 7. Fifty-four international HPB surgeons received an invitation to participate in the survey, of whom 31 (57 per cent) completed part I of the survey. Responses came from Europe ($n = 27$ respondents), Asia ($n = 2$), and North America ($n = 2$) (a complete list of respondents is provided in Appendix 8). The number of respondents agreeing with the proposed definitions is depicted in Table 3.3. Of important notice, less than two-thirds of the respondents agreed with the proposed definitions of operative mortality and intra-abdominal bleeding.

C. Consensus-based definitions of complications of liver surgery

After 2 consensus meetings with the project group, proposed definitions of complications of liver surgery were composed. The definitions of operative mortality and intra-abdominal bleeding were considerably changed after critical review. In line with suggestions of the respondents, intra-abdominal bleeding was divided into intra-abdominal hemorrhage and intra-abdominal hematoma. The final, proposed definitions are as follows:

- *Operative mortality*: death of a patient during or after the initial surgical procedure that is related to the surgical procedure.
- *Bile leakage:* any quantity of bile leaking via the abdominal wound or drains at least 48hr postoperatively; intra-abdominal collection of bile at the time of radiological imaging, re-operation or percutaneous drainage; cholangiographic evidence of contrast leakage. Fluid in drain or intra-abdominal collection should have a bilirubin content at least three-times the upper normal serum level in patients with postoperatively normal serum bilirubin levels, or a 50 per cent higher bilirubin level than

Table 3.3 Number of respondents agreeing with the proposed definition of clinical outcomes of liver surgery.

Clinical outcome	***N* = 31**
Operative mortality	19 (61)
Bile leakage	22 (71)
Blood transfusion	27 (87)
Intra-abdominal abscess	26 (84)
Wound infection	28 (90)
Intra-abdominal bleeding	14 (45)
Post-resectional liver failure	21 (68)
Pneumonia	29 (94)
Ascites	23 (74)
Pleural effusion	25 (81)
Sepsis	30 (97)
Acute renal failure	28 (90)

The cutoff level for approval was set at more than two-thirds of the respondents indicating to agree with the proposed definition; Numbers between parentheses are percentages; *N*, number.

the serum bilirubin level in patients with postoperatively elevated serum bilirubin levels.

→ Additional information: 16 respondents (52 per cent) believed that biochemical evidence of bilirubin in the drain fluid was not a prerequisite for the diagnosis bile leakage. In accordance, 17 respondents (55 per cent) did not routinely check bilirubin levels in drain fluid.

- *Blood transfusion:* administration of any quantity of red blood cells during the operation or within 48hr after surgery.

  → Additional information: 16 respondents (52 per cent) indicated that not only intra-operative blood transfusions, but also administration of red blood cells after surgery (within the first 24 to 48hr) should be recorded.
- *Intra-abdominal abscess*: any quantity of purulent fluid leaking via the abdominal drain; walled-off collection of pus in the abdominal cavity at the time of radiological imaging, re-operation or percutaneous drainage. Fluid in drain or intra-abdominal collection should be culture-positive.

  → Additional information: 74 per cent of respondents (*n* = 23 respondents) indicated that a microbiological culture of the fluid should be positive, unless antibiotics had been given empirically in advance of a microbiological culture.
- *Intra-abdominal hemorrhage:* any quantity of blood leaking via the abdominal drain either or not accompanied by a drop in hemoglobin level; intra-abdominal active bleeding during radiological imaging or re-operation.
- *Intra-abdominal hematoma:* collection of blood in the proximity of the liver that can be diagnosed either radiologically or during re-operation.

- *Post-resectional liver failure:* postoperative acquired failure of one or more of the hepatic excretory, detoxifying and synthetic functions that include serum bilirubin of more than 50 µmol/L [> 2.9 mg/dL], a prothrombin index below 50 per cent [international standardized ratio > 1.7] and/or hepatic encephalopathy grade 3 or 4 from postoperative day 3 onwards. Other obvious causes for the observed deterioration should be excluded (e.g. biliary obstruction).
  → Additional information: according to the respondents, additional parameters suggestive of hepatic failure were changes in serum ammonia, lactate or albumin levels.
- *Ascites:* pathological accumulation of clear fluid in the abdominal cavity evidenced by leakage of fluid in an amount of more than 500mL/day via the abdominal wound or, if present, the abdominal drain from postoperative day 3 onwards.
  → Additional information: the majority of respondents (74 per cent) indicated not to check the nature of the fluid in order to differentiate between transudate and exudate.
- *Pleural effusion:* any fluid in the pleural cavity proven by radiological imaging
  → Additional information: 25 out of 31 (82 per cent) respondents considered asymptomatic pleural effusion a normal response to liver surgery.

The majority of respondents agreed with the definitions of wound infection, pneumonia and sepsis, described by the Centres for Diseases Control and Prevention[63], and acute renal failure, described by Singri and colleagues[64], which are as follows:

- *Wound infection[63]:* divided into superficial and deep incisional surgical site infection (SSI):
  - Superficial incisional SSI
  Infection occurs within 30 days after the operation and infection involves only skin or subcutaneous tissue of the incision and at least one of the following:
  1. Purulent drainage, with or without laboratory confirmation, from the superficial incision.
  2. Organisms isolated from an aseptically obtained culture of fluid or tissue from the superficial incision.
  3. At least one of the following signs or symptoms of infection: pain or tenderness, localized swelling, redness, or heat, and superficial incision is deliberately opened by surgeon, unless incision is culture-negative.
  4. Diagnosis of superficial incisional SSI by the surgeon or attending physician.
  - Deep incisional SSI
  Infection occurs within 30 days after the operation and the infection appears to be related to the operation and infection involves deep soft tissues of the incision and at least one of the following:

1. Purulent drainage from the deep incision but not from the organ / space component of the surgical site.

2. A deep incision spontaneously dehisces or is deliberately opened by a surgeon when the patient has at least one of the following signs or symptoms: fever (temperature of more than 38°C), localized pain, or tenderness, unless site is culture-negative.

3. An abscess or other evidence of infection involving the deep incision is found on direct examination, during re-operation, or by histopathological or radiologic examination.

4. Diagnosis of a deep incisional SSI by a surgeon or attending physician.

- *Pneumonia*[63]*:* clinical entity meeting one of the following criteria:

1. Rales or dullness to percussion on physical examination of the chest and any of the following:

a. New onset of purulent sputum or change in character of sputum.

b. Organisms isolated from blood culture.

c. Isolation of pathogens from specimen obtained by transtracheal aspirate, bronchial brushing, or biopsy.

2. Chest radiographic examination shows new or progressive infiltrate, consolidation, cavitation, or pleural effusion and any of the following:

a. New onset of purulent sputum or change in character of sputum.

b. Organisms isolated from blood culture.

c. Isolation of pathogens from specimen obtained by transtracheal aspirate, bronchial brushing, or biopsy.

d. Isolation of virus or detection of viral antigen in respiratory secretions.

e. Diagnostic single antibody titer (IgM) or fourfold increase in paired serum samples (IgG) for pathogen.

f. Histopathological evidence of pneumonia.

- *Sepsis*[63]*:* the clinical syndrome defined by the presence of both infection and a systemic inflammatory response.

- Infection: pathological process caused by invasion of normally sterile tissue or fluid or body cavity by pathogenic or potentially pathogenic micro-organisms.

- Systemic inflammatory response syndrome (at least 2 criteria must be met):

1. Body temperature higher than 38°C or lower than 36°C

2. Heart rate higher than 90/min

3. Hyperventilation evidenced by respiratory rate higher than 20/min or arterial carbon dioxide partial pressure lower than 32 mmHg

4. White blood cell count higher than 12,000 cells/μL or lower than 4,000 cells/μL

- *Acute renal failure*[64]*:* increase in serum creatinine of 0.5 mg/dL [44.2 μmol/L] if baseline is below 2.5 mg/dL [221 μmol/L] or an increase in serum creatinine by more than 20 per cent if baseline level is above 2.5 mg/dL [221 μmol/L].

DISCUSSION

Clear definitions of clinical outcomes, used as primary or secondary endpoints of RCTs in liver surgery, are not commonly provided. If present, definitions differ substantially among trial reports, which hampers the interpretation of trial results and compromises the validity of systematic reviews and meta-analyses. The present study was designed to develop standard definitions of clinical outcomes of liver surgery. Based on a systematic literature search and consensus among international experts in HPB surgery, definitions of clinical outcomes of liver surgery are proposed.

Consistency in study design and adequacy in study reporting are essential to allow accurate interpretation of study data and comparison of study results. Examples of initiatives leading to more uniform reporting in HPB surgery are, for example, the *Brisbane 2000* system of the nomenclature of hepatic anatomy and resection[13], the recommendation of endpoints for clinical trials in hepatocellular carcinoma[12] and the Clavien-Dindo severity grading system[65]. The present systematic literature search demonstrated that consistency was lacking with regard to definitions of clinical endpoints of RCTs in liver surgery. Inconsistent definitions are undesirable as they are not reproducible. The conduct of systematic reviews and meta-analyses is hindered by the lack of consistency, while combining results from different RCTs is becoming increasingly important in the field of HPB surgery. This may be due to the low feasibility of conducting adequately powered RCTs using single component, clinical endpoints as primary endpoints as a consequence of declining event rates after liver surgery[5, 66].

The present comprehensive literature search showed that the mean sample size of RCTs in liver surgery was 100 patients. A previous study demonstrated that, due to the low rates of complications of liver surgery, sample sizes ranging between 826 and 6 250 patients per study arm would be needed for an adequately powered RCT aiming to show a 50 per cent relative reduction of a single component, primary endpoint[5]. As a consequence, many of the RCTs included in the present systematic review are underpowered with respect to single component, clinical outcomes and their conclusions should be interpreted with caution. Problems with the quality and sample size of RCTs in liver surgery have been reported before[8, 67, 68]. Larger sample sizes can be obtained by the conduct of multicentre trials, institution of national databases and performance of meta-analyses[1, 69].

The implementation of a liver surgery specific CEP might enhance the feasibility of liver surgery-related RCTs using clinical outcomes as primary endpoint. A CEP increases the statistical power of an RCT by capturing the number of patients experiencing any one of several clinical outcomes within a specific timeframe[70, 71]. To the best of our knowledge, a well-defined CEP to be used as primary endpoint of RCTs in liver surgery is lacking. A first step in the design of a CEP is achieving consensus on definitions of its

components. The definitions proposed in the present paper can be used to accurately describe the components of a future liver surgery specific CEP.

Simultaneously to the efforts of our group, the International Study Group of Liver Surgery (ISGLS) developed consensus definitions of the complications post-hepatectomy liver failure, bile leakage and post-hepatectomy hemorrhage[72-74]. The definitions proposed by the ISGLS resemble those presented in the present paper. An advantage of the ISGLS definitions is their retrospective validation in large patient cohorts. On the other hand, a remarkable difference exists between their work and the present study concerning the aspect of severity grading. Stratification of the severity of complications is important as it allows for judicious interpretation of data. Based on the results of our survey and preference of the respondents, the objective Clavien-Dindo classification for severity grading of surgical complications was selected[15, 75, 76], whereas the ISGLS proposes to use a newly designed classification system. The Clavien-Dindo severity grading system is widely used in surgical literature, allows for objective grading of complications, and reflects the severity of a complication from the doctors' and patients' perspective[65]. The grading system proposed by the ISGLS may introduce confusion as it does not adhere to the Clavien-Dindo grades, which are familiar to most HPB surgeons. Moreover, it introduces subjectivity by, for example, stating that Grade A bile leakage implies "no or little change in patients' clinical management"[72]. In order to use and interpret CEPs accurately, objective severity grading is important as it has been proven that CEPs can be misleading if components vary widely in clinical relevance[7, 14, 77]. In fact, components with low clinical relevance tend to have a higher event rate and treatment effect[14].

To conclude, the proposed definitions of clinical outcomes of liver surgery can be used to uniformly describe clinical endpoints of RCTs. The presence of standard definitions of complications of liver surgery enables the conduct of systematic reviews and meta-analyses in HPB surgery and forms the first step in the process of developing a well-defined liver surgery specific CEP. Selection of components of a future liver surgery specific CEP is the next challenge to meet.

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Chapter 4

Development of a composite endpoint for randomized controlled trials in liver surgery

Adapted from:

van den Broek MA, van Dam RM, van Breukelen GJ, Bemelmans MH, Oussoultzoglou E, Pessaux P, Dejong CH, Freemantle N, Olde Damink SW. Development of a composite endpoint for randomized controlled trials in liver surgery. *Br J Surg* 2011; 98: 1138-45.

ABSTRACT

Background

The feasibility of randomized controlled trials (RCTs) in liver surgery using a single component, clinical endpoint is low as such endpoints require large sample sizes owing to their low incidence. A liver surgery specific composite endpoint (CEP) could solve this problem. The aim of this study was to develop a liver surgery specific CEP.

Methods

Components of a liver surgery specific CEP were selected based on a systematic literature search and consensus among international HPB surgeons. As an example, two prospective cohorts of patients who had undergone liver surgery in high-volume HPB centres were used to assess the event rate of the proposed liver surgery specific CEP and effect of its implementation on the theoretical sample size of a future RCT.

Results

Components selected for the liver surgery specific CEP were ascites, post-resectional liver failure, bile leakage, intra-abdominal hemorrhage, intra-abdominal abscess and operative mortality, all with a Clavien-Dindo grade of 3 or more and occurring within 90 days after initial liver surgery. The incidence of this liver surgery specific CEP was 19 per cent in one cohort and 11 per cent in the other. These rates led to an approximate twofold reduction in the sample size required for an adequately powered RCT in liver surgery using the liver surgery specific CEP instead of a single component, clinical endpoint as primary endpoint.

Conclusion

The proposed liver surgery specific CEP consists of ascites, post-resectional liver failure, bile leakage, intra-abdominal hemorrhage, intra-abdominal abscess and operative mortality. It has a considerably higher event rate than any of its individual components. Its use as a primary endpoint will increase the feasibility and comparability of RCTs in liver surgery.

INTRODUCTION

In the current era of evidence-based practice, randomized controlled trials (RCTs) deliver the highest level of evidence. Therefore, their conclusions are frequently incorporated into treatment guidelines. Few RCTs have been performed in liver surgery[1]. This low number may be explained by the large sample size needed for an adequately powered trial using clinically relevant primary endpoints such as liver surgery-related mortality or specific types of morbidity[2]. These large sample sizes present a dilemma to clinical investigators who want to assess the efficacy of interventions in liver surgery on single component, clinical outcomes.

The primary endpoint of an RCT is dependent on its objective and must be chosen carefully *a priori*[3]. This is particularly important as it is the primary endpoint alone that should be used to judge whether a treatment has been effective in a trial[4]. Clinical outcomes such as surgery-related mortality or specific types of morbidity are important for the evaluation of the efficacy of surgical interventions. However, they are of limited use in future RCTs in liver surgery as their event rate is so low that large sample sizes are needed for adequately powered trials[2, 5]. A possible solution would be the implementation of a well-defined composite endpoint (CEP) to be used as the primary endpoint of RCTs in liver surgery.

A CEP is a combination of two or more procedure specific complications that are considered as a single, dichotomous outcome[6]. CEPs lead to an increased statistical power and thus efficiency because their event rate is higher than that of a single component, clinical endpoint. Moreover, CEPs enhance the comparability of trial results, which facilitates meta-analyses, and take into account multiple outcomes or even multiple types of outcomes (for example patient-reported and clinical endpoints), thereby evaluating the overall benefit of a single intervention[7]. In order to do so, the individual components of a CEP need to be well-defined and clinically relevant outcome parameters.

Hitherto, CEPs have hardly been used as the primary endpoint of RCTs in liver surgery, and uniformity in components is lacking. The aim of the present study was to develop a standardized liver surgery specific CEP that can be used as primary endpoint of RCTs in liver surgery.

METHODS

A three-step approach was applied for the development of a liver surgery specific CEP. First, a systematic review was undertaken to identify frequently used dichotomous, clinical endpoints of RCTs in liver surgery (development step 1). These endpoints formed the basis of a web-based survey that aimed at reaching consensus on the definition of

complications of liver surgery (development step 2) and at selecting components of the liver surgery specific CEP (development step 3). Development steps 1 and 2 have been described in chapter 3 of the present thesis.

The present paper deals with the selection of components of the liver surgery specific CEP (development step 3). As an example, the event rate of the proposed liver surgery specific CEP was assessed in two prospective cohorts of patients who had undergone liver surgery in high-volume European HPB centres, and the theoretical sample size of an RCT using the liver surgery specific CEP as the primary endpoint was calculated.

Development step 1. Identification of single component, clinical endpoints of randomized controlled trials in liver surgery

An electronic search was performed to identify all single component, clinical outcomes used as primary or secondary endpoints of RCTs in liver surgery published between 1 January 2004 and 31 December 2008. The results if this search have been presented in chapter 3 of the present thesis.

Development step 2. Consensus on definitions of complications of liver surgery

The endpoints most frequently used in the identified RCTs formed the basis of a web-based survey. An example of the survey can be found in Appendix 7. The survey consisted of two parts. Definitions of complications of liver surgery were proposed in part I of the survey (described in chapter 3 of the present thesis).

Development step 3. Selection of components of a liver surgery specific CEP

Part II of the survey consisted of questions on possible components of the liver surgery specific CEP. Complications preferred for inclusion in the liver surgery specific CEP by at least two-thirds of respondents were considered as components. For the final selection, additional important attributes of CEP components were taken into account[7, 8]. In short, each component of the CEP should preferably (1) be easily ascertainable (i.e. presence of a standardized definition), (2) be of substantial clinical relevance (i.e. reflecting Clavien-Dindo severity grade 3 to 5[9]), and (3) have the same expected direction of treatment effect.

Evaluation of the event rate of the liver surgery specific CEP

The event rate of the individual components and the liver surgery specific CEP was determined using two prospective databases consisting of consecutive patients who had undergone partial liver resection between January 2000 and October 2009. These data sets were derived from the Department of Surgery of Maastricht University Medical Centre (Maastricht, the Netherlands) and the Division of HPB Surgery of Hautepierre University Hospital (Strasbourg, France), both high-volume European HPB centres.

Patients who had concomitant gastrointestinal procedures or bilio-enteric anastomoses were excluded. The occurrence of an event within 90 days of initial liver surgery was determined and graded using the Clavien-Dindo classification[9].

Statistical analysis and sample size calculation

Continuous data were expressed as mean (standard deviation) when normally distributed and median (range) when non-normally distributed. SPSS version 15.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

The theoretical sample size of an adequately powered RCT was calculated as the minimum number of patients necessary in each arm of the trial, with $\alpha = 0.05$ and $\beta = 0.20$, using two-sided testing. The formula used for sample size calculations can be found in Appendix 2. The sample size was calculated assuming a one-third, one-half or two-thirds relative reduction in the incidence of either a single component endpoint or the proposed liver surgery specific CEP.

RESULTS

Development step 1. Identification of single component, clinical endpoints of RCTs in liver surgery

The electronic search identified 326 abstracts that fulfilled the search terms. After selection, 47 RCTs were included in the final analysis (reference list and study details are provided in chapter 3 of the present thesis and Appendix 5). Eighteen RCTs (38 per cent) had a single component, dichotomous primary endpoint and twenty-nine (62 per cent) a surrogate, continuous primary endpoint. Eight RCTs (17 per cent) used a CEP as primary endpoint, which consisted of postoperative complications in 4 RCTs, and overall intra-abdominal complications, ischemia-related complications, abdominal cavity-related complications and surgical site infections in 1 RCT each (Appendix 5). The twelve single component, clinical endpoints most frequently used in RCTs in liver surgery were: operative mortality ($n = 38$), bile leakage ($n = 35$), blood transfusion ($n = 34$), intra-abdominal abscess ($n = 28$), wound infection ($n = 27$), intra-abdominal hemorrhage ($n = 25$), post-resectional liver failure ($n = 24$), pneumonia ($n = 23$), ascites ($n = 21$), pleural effusion ($n = 20$), sepsis ($n = 9$), and acute renal failure ($n = 7$). These complications formed the basis of part I of the electronic survey.

Development step 2. Consensus on definitions of complications of liver surgery

Fifty-four HPB surgeons received an invitation to participate in the survey, of whom 31 (57 per cent) completed part I of the survey on definitions of complications of liver surgery. A complete list of respondents is provided in Appendix 8. The proposed defini-

Table 4.1 Selection of components of the liver surgery specific composite endpoint.

Complication	*N* respondents considering the complication liver surgery specific (*n* = 31)	*N* respondents indicating the complication should be included in the liver surgery specific CEP (*n* = 28)
Pneumonia	1 (3)	12 (43)
Wound infection	3 (10)	7 (25)
Pleural effusion	18 (58)	9 (32)
Blood transfusion	10 (32)	20 (71)
Intra-abdominal abscess	13 (42)	20 (71)
Intra-abdominal hemorrhage	13 (42)	23 (82)
Bile leakage	31 (100)	28 (100)
Ascites	23 (74)	25 (89)
Sepsis	3 (10)	17 (61)
Post-resectional liver failure	29 (94)	28 (100)
Acute renal failure	3 (10)	14 (50)
Operative mortality	n.a.	24 (86)

Numbers in parentheses are percentages; *N*, number; CEP, composite endpoint; n.a., not applicable.

tions of the most frequently used complications have been described in chapter 3 of the present thesis. Bile leakage, post-resectional liver failure and ascites were regarded as liver surgery specific complications by 31, 29 and 23 respondents, respectively (Table 4.1).

Development step 3. Selection of components of the liver surgery specific CEP

Twenty-eight (52 per cent) HPB surgeons completed part II of the survey. Their preferences regarding the components of the liver surgery specific CEP are shown in Table 4.1. As a result, six complications with Clavien-Dindo grade 3 or more and preferred by at least two-thirds of the respondents were selected as components: ascites, post-resectional liver failure, bile leakage, intra-abdominal abscess, intra-abdominal hemorrhage, and operative mortality. Although blood transfusion had a preference rate of 71 per cent, it was not included as it is regarded as a complication with a Clavien-Dindo grade of less than 3. All respondents indicated that they would be interested in using a liver surgery specific CEP as primary endpoint of an RCT.

Evaluation of the event rate of the liver surgery specific CEP

Between January 2000 and October 2009, 340 and 583 adult patients underwent partial liver resection at Maastricht University Medical Centre and University Hospital of Strasbourg, respectively. Of these, 49 and 69 patients were excluded because of concomitant gastrointestinal procedures or bilio-enteric anastomoses. Consequently, 291 and 514

Table 4.2 Patient characteristics and event rates of complications of liver surgery at Maastricht University Medical Centre and University Hospital of Strasbourg.

	Maastricht University Medical Centre (*n* = 291)	**University Hospital of Strasbourg (*n* = 514)**
Sex (female : male)	133 : 158	229 : 285
Age (years)*	60.0±12.8	59.5±12.6
Major hepatectomy†	114 (39.2)	253 (49.2)
Complication grade 1-2‡	**Incidence (%)**	**Incidence (%)**
Ascites	2 (0.7)	22 (4.3)
Post-resectional liver failure	8 (2.7)	11 (2.1)
Bile leakage	1 (0.3)	7 (1.4)
Intra-abdominal abscess	2 (0.7)	6 (1.2)
Intra-abdominal hemorrhage	1 (0.3)	0 (0)
Complication grade 3-5‡	**Incidence (%)**	**Incidence (%)**
Ascites	9 (3.1)	1 (0.2)
Post-resectional liver failure	10 (3.4)	18 (3.5)
Bile leakage	35 (12.0)	23 (4.5)
Intra-abdominal abscess	30 (10.3)	23 (4.5)
Intra-abdominal hemorrhage	3 (1.0)	5 (1.0)
Operative mortality	10 (3.4)	7 (1.4)
Liver surgery specific CEP	56 (19.2)	55 (10.7)

Numbers in parentheses are percentages; * values are mean ± standard deviation; † resection of three or more liver segments; ‡ according to the Clavien-Dindo classification[9]; *N*, number; CEP, composite endpoint.

patients were included in the present analysis. Their characteristics and postoperative complication rates are depicted in Table 4.2.

At Maastricht University Medical Centre, the rates of the individual components of the proposed liver surgery specific CEP with Clavien-Dindo grade 3 or more within 90 days of initial surgery ranged from 1.0 per cent for intra-abdominal hemorrhage to 12.0 per cent for bile leakage; the mortality rate was 3.4 per cent. The incidence of the liver surgery specific CEP was 19.2 per cent (*n* = 56). The rates of the individual components of the liver surgery specific CEP at the University Hospital of Strasbourg ranged from 0.2 to 4.5 per cent. Fifty-five patients (10.7 per cent) experienced at least one of the six complications of the liver surgery specific CEP (Table 4.2).

Based on the rates of complications in the two data sets, the sample size of a single-centre RCT intending to show a relative reduction in either the individual complication or the liver surgery specific CEP by one-third, one-half or two-thirds was calculated (Table 4.3).

Table 4.3 Sample size of each arm of an adequately powered randomized controlled trial aiming to show a one-third, one-half or two-thirds relative reduction of the primary endpoint.

	Maastricht University Medical Centre			University Hospital of Strasbourg		
	Relative reduction			Relative reduction		
Complication	**33%**	**50%**	**66%**	**33%**	**50%**	**66%**
Ascites	3 695	1 481	742	58 701	23 484	11 744
Post-resectional liver failure	3 360	1 348	675	3 261	1 308	656
Bile leakage	881	356	180	2 515	1 009	506
Intra-abdominal abscess	1 043	421	212	2 515	1 009	506
Intra-abdominal hemorrhage	11 661	4 668	2 336	11 661	4 668	2 336
Operative mortality	3 360	1 348	675	8 301	3 324	1 664
Liver surgery specific CEP	514	209	106	1 000	404	203

Assumptions $\alpha = 0.05$ and $\beta = 0.20$ and two-sided testing; CEP, composite endpoint.

DISCUSSION

The use of a CEP as primary endpoint of RCTs in liver surgery is not very common at present and uniformity of CEP components is lacking. In this study, a consensus-based and standard liver surgery specific CEP was developed after a systematic literature search and survey among international experts in liver surgery. The proposed liver surgery specific CEP includes ascites, post-resectional liver failure, bile leakage, intra-abdominal hemorrhage, intra-abdominal abscess, and operative mortality, all occurring within 90 days of initial surgery and with Clavien-Dindo severity grade of at least 3. The event rate of the liver surgery specific CEP was substantially higher than that of its individual components. As a consequence, the theoretical sample size required for an adequately powered RCT using the liver surgery specific CEP as primary endpoint decreased considerably when tested in two patient cohorts from high-volume HPB centres in Europe. Implementation of the liver surgery specific CEP will increase the feasibility of RCTs that use clinical outcomes of liver surgery as primary endpoints.

Investigators in medical disciplines other than liver surgery frequently choose CEPs as the primary endpoint of their RCTs[10-12]. Joint efforts in these fields have already led to the formulation of preferred characteristics of CEP components[7, 8]. It has been shown that the use of CEPs can be misleading if components are ill-defined or vary widely in clinical relevance or expected direction of treatment effect[6, 10, 13, 14]. As a consequence, careful selection of CEP components is warranted.

In order to overcome these demerits of CEPs, the aforementioned pitfalls were avoided systematically. First, international consensus on the definitions of complications of liver surgery was reached by a web-based survey. Second, all complications included in the liver surgery specific CEP had to be classified as Clavien-Dindo grade 3 or more because

this ensures genuine clinical relevance. The Clavien-Dindo classification has been designed to categorize complications of surgery objectively, based on a therapy-oriented severity grade[9]. Although this system basically focuses on a medical perspective, it has recently been proven also to reflect severity from the patients' perspective[15]. Finally, interpretation of the effect size of an intervention and its direction on the individual components is facilitated by adequate reporting of the incidence of all components during the total observation period[6, 7, 10]. Statistical analysis of these secondary outcomes should be performed using adequate consideration of α spending and the question of multiple testing[3, 16]. It is important to appreciate that secondary outcomes, even if nominally significant, are of secondary importance and not decisive in favour of one treatment if the primary endpoint fails to show a significant effect[4].

Only eight of 47 RCTs used a CEP as primary endpoint, of which four defined the CEP as "postoperative complications". The use of postoperative complications as CEP is confusing as it is unclear which individual complications account for its incidence. It has been proven that clinically irrelevant complications can contribute greatly to the event rate of such CEPs[10]. Therefore, explicit statement of CEP components and assurance of their clinical relevance are essential.

The effect of introducing a liver surgery specific CEP was assessed using two patient cohorts. The complication rates in both cohorts were within the 95 per cent prediction intervals that were calculated in a recent meta-analysis on complications of liver surgery[2]. Therefore, these two cohorts were considered representative of current HPB practice. The incidence of the liver surgery specific CEP was 10.7 per cent in the Strasbourg and 19.2 per cent in the Maastricht cohort. The difference between the two data sets might be explained by differences in surgical technique and perioperative care or, alternatively, by differences in data registration and definitions of complications. Adoption of standard registration methods and uniform definitions of complications of liver surgery can decrease the latter type of heterogeneity. The American College of Surgeons National Surgical Quality Improvement Programme could serve as an example of standardized reporting of outcomes of HPB surgery (www. acsnsqip.org)[17].

A drawback of this study is the inevitable selection bias introduced by the application of a survey on invitation. HPB surgeons from all continents were invited to complete the survey. The majority of responses came from Europe, and therefore, the results of the survey represent the current view on trial design in the European HPB community. An open survey is currently under development in order to collect a broader opinion of the HPB community on definitions and components of the liver surgery specific CEP. As it might prove difficult to use the liver surgery specific CEP as primary endpoint for all RCTs in liver surgery, it may be necessary to design CEPs customized for trials focussing on interventions with medication or surgical technique. Furthermore, assigning weights to components based on their clinical impact or expression of the liver surgery specific CEP

on an ordinal six-point scale may be considered in the future. However, as the majority of patients in both institutes had an uneventful clinical course, only marginal gain of power should be expected from the latter.

Prospective validation of the proposed liver surgery specific CEP, using data from institutes originating from all continents collected by well-trained research assistants using uniform definitions, is warranted.

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Part II

Assessing outcomes of liver surgery: hepatic damage as a result of surgical technique

Chapter 5

Liver manipulation during liver surgery in humans is associated with hepatocellular damage and hepatic inflammation

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ABSTRACT

Background

Manipulation of the liver during liver surgery results in profound hepatocellular damage. Experimental data show that mobilization-induced hepatocellular damage is related to hepatic inflammation. To date, information on this link in humans is lacking. As it is possible to modulate inflammation, it is clinically relevant to unravel this relation. This observational study aimed to establish the association between liver mobilization and hepatic inflammation in humans.

Methods

Consecutive patients requiring mobilization of the right hemi-liver during liver surgery were studied. Plasma samples and liver biopsies were collected prior to and directly after mobilization and after transection of the liver. Hepatocellular damage was assayed by liver fatty acid-binding protein (L-FABP) and aminotransferase levels. Hepatic inflammation was determined by (a) immunohistochemical identification of myeloperoxidase (MPO) and CD68- positive cells and (b) hepatic gene expression of inflammatory and cell adhesion molecules (IL-1β, IL-6, IL-8, VCAM-1 and ICAM-1).

Results

A total of 25 patients were included. L-FABP levels increased significantly during mobilization (301±94 ng/mL to 1599±362 ng/mL, p=0.008), as did ALAT levels (36±5 IU/L to 167±21 IU/L, p<0.001). A significant increase in MPO (p=0.001) and CD68 (p=0.002) positive cells was noticed in the liver after mobilization. The number of MPO-positive cells correlated with the duration of mobilization (Pearson correlation=0.505, p=0.033). Hepatic gene expression of pro-inflammatory cytokines IL-1β and IL-6, chemo-attractant IL-8 and adhesion molecule ICAM-1 increased significantly during liver manipulation.

Conclusion

Liver mobilization is associated with hepatocellular damage and liver inflammation, as shown by infiltration of inflammatory cells and upregulation of genes involved in acute inflammation.

INTRODUCTION

Surgical resection is the ultimate treatment for a variety of benign and malignant liver tumours. During liver surgery, there is a delicate balance between the attempt to achieve surgery with curative intent and the necessity to leave adequate remnant liver volume in order to avoid post-resectional liver failure (PLF). Risk factors for the development of PLF may either be surgery- or patient-related[1].

With respect to surgery-related risk factors, excessive intra-operative blood loss and the need for blood transfusion are associated with adverse post-resectional outcomes[2]. In order to limit blood loss during liver surgery, different surgical techniques have been introduced. One of these techniques is mobilization of the liver prior to transection. During mobilization, the liver is forcefully manipulated in order to dissect its ligaments and control direct venous branches to the inferior caval vein. Recent data deliver convincing evidence that mobilization of the liver in itself causes substantial hepatocellular injury[3-7]. The highly sensitive liver cell damage markers liver fatty acid-binding protein (L-FABP), arginase-1, glutathione-s-transferase-α and cell-free circulating albumin-mRNA increased significantly during mobilization of the liver and did not increase thereafter during either inflow occlusion or transection[3-7]. Of important notice, manipulation-induced liver cell damage negatively affected postoperative outcomes in patients undergoing liver surgery for hepatocellular carcinoma[7].

The pathogenesis of mobilization-induced liver damage has been studied in detail in a murine model of liver transplantation[8-10]. In short, mobilization of the liver induced neuronal mediated disturbances in the hepatic microcirculation leading to both liver cell damage and hepatic inflammation. Activated Kupffer cells seemed to play a central role as modulation of their function largely prevented hepatocellular damage and improved experimental outcome[11]. In man, it has been shown that systemic inflammation, reflected by plasma interleukin-6 (IL-6) levels, followed liver manipulation during surgery[3]. However, the source of this systemic inflammatory response is yet unidentified in humans. Based on aforementioned experimental observations, hepatic inflammation might well be involved in the cascade of manipulation-induced liver cell damage and systemic inflammation.

Given the possibility to modulate the inflammatory response, identification of the link between manipulation-induced liver cell injury and inflammation in man could identify novel therapeutic strategies for its prevention. This study aimed to establish the association between liver mobilization, hepatocellular damage and hepatic inflammation in patients undergoing liver surgery.

METHODS

Patients

Consecutive patients scheduled to undergo liver surgery requiring full mobilization of the right hemi-liver at Maastricht University Medical Centre between October 2007 and June 2009 were included in this observational study. Exclusion criteria were (a) the presence of cirrhosis of the liver confirmed by preoperative liver biopsy, (b) repeat liver surgery, (c) laparoscopic liver surgery, (d) use of anti-inflammatory drugs, (e) presence of renal failure (defined as serum creatinine above 137 µmol/L in males and above 104 µmol/L in females[12]), (f) performance of an extra-hepatic procedure, and (g) participation in another trial.

Resections were divided into major (3 or more Couinaud segments) or minor (less than 3 Couinaud segments or non-anatomical wedge resections)[13]. All data were prospectively entered in a database and the clinical course of the participants was studied up until 90 days after discharge. The incidence rate of the liver surgery specific composite endpoint and its individual components (ascites, bile leakage, intra-abdominal hemorrhage, intra-abdominal abscess, PLF and operative mortality) was calculated[14]. The study was approved by the Medical Ethics Committee of Maastricht University Medical Centre and all participating individuals gave written informed consent.

Surgical procedure

Patients routinely had two peripheral venous catheters and indwelling catheters in a jugular vein and radial artery. Immediately preoperatively, all patients received a single intravenous dose of 2200 mg amoxicillin/clavulanic acid as antibiotic prophylaxis. Propofol and isoflurane were used as anaesthetics. Surgical procedures were commenced using a subcostal bilateral incision as described earlier[5]. An Omni-Flex General Retractor System (Integra LifeSciences Corporation, Plainsboro, NJ, USA) was used to improve exposure. After dissection of the teres hepatis ligament, the procedure was continued with dissection of the falciform ligament and further mobilization of the right hemi-liver from the posterior abdominal wall. Thereafter, the liver was rotated anteriorly and to the left in order to dissect direct venous branches to the inferior caval vein. Full mobilization was reached when the caval vein was dissected free of all its attachments at the 12 o'clock anterior surface. Subsequently, an intra-operative ultrasound was performed which directed the surgical strategy. A Cavitron Ultrasonic Surgical Aspirator (CUSA system 200 macrodissector, Cavitron Surgical Systems, Stamford, CT, USA) and Argon beam coagulation (Force GSU System, Valleylab, Boulder, CO, USA) were used for liver transection. Inflow occlusion was not routinely applied. If necessary, a complete or selective Pringle maneuver (with 15 min or 30 min ischemic cycles) or ligation of the appropriate portal pedicle vessels was applied[5]. During transection, central venous pressure was

maintained below 5 mmHg. Postoperative care was provided according to an enhanced recovery after liver surgery programme[15].

Blood and tissue sampling

Before, during and after the operative procedure, arterial blood was drawn from the radial artery catheter according to a predetermined protocol at different time points (Figure 5.1). Blood samples were transferred to prechilled EDTA tubes and subsequently centrifuged at 4°C at 3500 x g for 15 min. Plasma was stored at −80°C until batch analysis.

Liver wedge biopsies were taken using scissors at fixed time points during the procedure from segment 5 of the liver. The first liver wedge biopsy was obtained immediately after opening of the abdomen and before touching or manipulating the intestines or liver, the second biopsy was collected after full mobilization of the right hemi-liver and before application of inflow occlusion or liver transection, and the third after liver transection. Defined 0.5 x 0.5 cm fragments of liver tissue were cut, snap-frozen in liquid nitrogen and stored at −80°C. Fragments of the same size were immersed in Tissue-Tek optimal cutting temperature compound (Sakura Finetek Europe, Zoeterwoude, The Netherlands) and mounted on a piece of cork before they were frozen in prechilled isopentane on dry ice and stored at −80°C.

Hepatocellular damage

The extent of hepatocellular damage was assessed by plasma concentrations of L-FABP and aminotransferases. L-FABP is a sensitive marker for the detection of liver cell damage[16, 17]. L-FABP levels were determined using a commercially available ELISA (Hycult Biotechnology, Uden, the Netherlands). According to the manufacturer's manual, L-FABP plasma levels in healthy individuals were approximately 12 ng/mL. Alanine aminotransferase (ALAT) levels were assayed by the clinical chemistry laboratory of Maastricht University Medical Centre. The upper limit of normal was 35 IU/L.

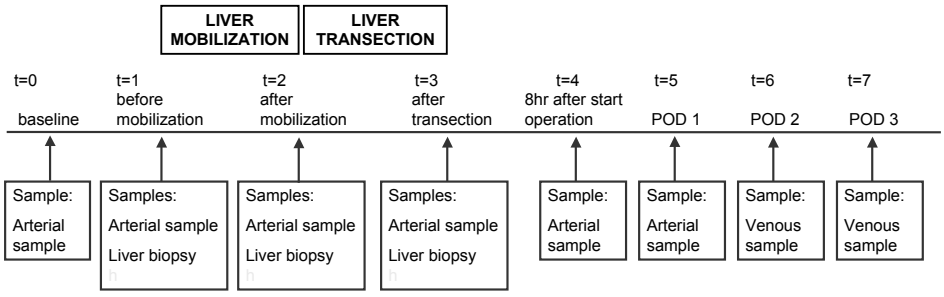

Figure 5.1 Flowchart of blood and tissue collection.
Legend: POD, postoperative day.

Immunohistochemistry of the liver

Tissue-Tek embedded frozen liver biopsies were cut in 7 µm sections, fixed in acetone for 10 min and subsequently blocked for endogenous peroxidase activity by incubation in 0.3 per cent H_2O_2 in PBS. Primary antibodies were applied for 1hr after blocking with 10 per cent serum. The following primary antibodies were used: (i) rabbit anti-human myeloperoxidase (MPO) antiserum (dilution 1:1000; Dako, Glostrup, Denmark) as marker for neutrophils and macrophages; (ii) anti-CD68 (clone Kp1, dilution 1:400; Dako, Glostrup, Denmark) specific for monocytes/macrophages; and (iii) anti-caspase-3-mediated cleavage generated neo-epitope of cytokeratin 18 (M30, dilution 1:50; Roche, Mannheim, Germany) specific for hepatocyte apoptosis, as described previously[18]. Secondary antibodies consisted of horseradish peroxidase-labeled goat-anti-rabbit IgG (1:500; Jackson immunoResearch, Suffolk, UK) for MPO-staining. For CD68 and M30-staining, biotinylated rabbit-anti-mouse IgG was applied as secondary antibody (1:300 and 1:500 dilution, respectively), and the StrepAB/HRP complex (Dako, Glostrup, Denmark) was used for signal enhancement. Staining was visualized by DAB followed by haematoxylin for nuclear counterstaining. The stained slides were photographed at 200x magnification using a Nikon digital camera DXM1200 and ACT-1 v2.63 software from Nikon Corporation. Cells were counted in 6 randomly selected microscopical views, and cell numbers were noted as cells/mm^2 for MPO and M30-staining and as 0-3 ordinal scale for CD68-staining. The number of CD68-positive cells was categorized as follows: 0 (none), 1 (few), 2 (moderate numbers), and 3 (many). In addition, the morphology of CD68-positive cells was graded on a 1-3 scale as follows: 1 (normal appearance), 2 (moderate enlargement), and 3 (substantial enlargement).

Gene expression of inflammatory mediators in the liver

Expression of genes encoding for inflammatory mediators and cell adhesion molecules was determined in liver biopsies taken at three time points during surgery (Figure 5.1). Genes of interest included interleukin 1 beta (IL-1β) and interleukin 6 (IL-6), both pro-inflammatory cytokines involved in macrophage activation, interleukin 8 (IL-8), a chemokine involved in recruitment of inflammatory cells, and vascular cell adhesion molecule (VCAM-1) and intercellular adhesion molecule (ICAM-1), important for adhesion and migration of inflammatory cells.

Hepatic gene expression was assessed by real-time quantitative polymerase chain reaction (PCR). Total RNA was isolated from snap-frozen liver samples by using Tri-reagent (Sigma-Aldrich, St. Louis, MO, USA). Reverse transcription was performed using the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA, USA). Real-time PCR was performed on a Bio-Rad MyIQ using IQ SYBR Green Supermix (Bio-rad, Hercules, CA, USA). Primers for target genes were developed using Primer Express version 2.0 (Applied Biosystems, Foster City, CA, USA). Sequences of the applied PCR primers are listed in Appendix 9. To

standardize for cDNA concentration in the samples, the housekeeping gene cyclophylin A (peptidylprolyl isomerase A) was used. For calculations of the initial amount of mRNA present in the sample, the relative standard curve method was used.

Statistical analysis

Data are given as mean and standard error of the mean or median with range, depending on the nature of the data. Differences in hepatocellular damage markers, number of MPO and M30-positive cells and hepatic gene expression between the three time points during liver surgery (before mobilization, after mobilization and after transection) were calculated using the paired sample t-test. For CD68 number and morphology, median values were compared using Wilcoxon's signed rank test. In addition, correlations between duration of mobilization, influx of inflammatory cells and hepatic gene expression were calculated. A p-value below 0.050 was considered statistically significant. Statistical analysis was performed using SPSS version 20 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Patient flow

Between October 2007 and July 2009, one-hundred and two patients were scheduled to undergo a partial hepatic resection. Of these, 25 patients fulfilled the inclusion criteria. Reasons for exclusion were: presence of underlying liver disease confirmed by preoperative liver biopsy ($n = 4$), repeat liver surgery ($n = 6$), laparoscopic liver surgery ($n = 7$), no informed consent ($n = 9$), participation in another trial ($n = 6$), no liver resection during surgery ($n = 15$), no formal mobilization of right hemi-liver ($n = 24$), and performance of an extra-hepatic procedure ($n = 6$).

Patient characteristics

Characteristics of the included patients are shown in Table 5.1. Liver surgery was performed because of benign hepatic disease in 1 and secondary hepatic malignancies in 24 patients, consisting of colorectal liver metastases in 23 and carcinoid metastases in 1 patient, respectively. Major liver resections were performed in 17 and minor in 8 patients, with a median number of 4 resected segments (range 1-4). Mean operative time was 213±11 min, of which 65±5 min were used for mobilization of the right hemi-liver and 94±8 min for transection of liver parenchyma. The extent of resection did not influence the duration of liver mobilization (70±6 min for major resections versus 53±8 min for minor resections, p=0.112). During transection, a Pringle maneuver was applied in 15 patients (60 per cent). Mean cumulative ischemia time in patients undergoing tran-

Table 5.1 Patient characteristics.

	***N* = 25**
Baseline characteristics	
Age (years)	61 ± 2
Sex (male)	17 (68)
BMI (kg/m^2)	26 ± 1
Primary disease	
Benign	1 (4)
Malignant	24 (96)
Preoperative laboratory tests	
ALAT (IU/L)	30 ± 4
Bilirubin (total) (μmol/L)	13 ± 1
Prothrombin time (sec)	11 ± 1
Creatinine (μmol/L)	87 ± 3
Operative variables	
Type of resection	
Right hepatectomy	12 (48)
Trisectionectomy	4 (16)
Segmentectomy	9 (36)
Median no. resected segments (range)	4 (1-4)
Duration of surgery (min)	213 ± 11
Mobilization time (min)	65 ± 5
Transection time (min)	94 ± 8
Pringle maneuver	15 (60)
Selective Pringle	7 (28)
Complete Pringle	8 (32)
Total blood loss (mL)	1002 ± 167
Postoperative outcome	
Liver surgery specific composite endpoint	4 (16)

Numbers indicate mean ± standard error or absolute number (percentage) unless otherwise indicated; *N*, number; BMI, body mass index; ALAT, alanine aminotransferase.

section with a complete Pringle maneuver was 55±7 min. For patients with a selective Pringle maneuver of the right hemi-liver, mean cumulative ischemia time was 30±4 min.

The incidence of the liver surgery specific composite endpoint was 16 per cent (4 out of 25 patients). The component accounting for this incidence was bile leakage in all 4 patients. The rates of PLF and operative mortality were zero.

Liver cell damage markers increase significantly after liver mobilization

To characterize liver cell damage secondary to liver manipulation, L-FABP and ALAT levels were measured. Mean arterial L-FABP levels increased significantly during mobilization

of the right hemi-liver (from 301±94 ng/mL to 1599±362 ng/mL, p=0.008) and did not increase significantly thereafter (2791±872 ng/mL, p=0.696 versus after mobilization), as depicted in Figure 5.2A. ALAT concentration also increased significantly during mobilization of the right hemi-liver (from 36±5 IU/L to 167±21 IU/L, p<0.001) and further increased during transection (408±61 IU/L, p<0.001 versus after mobilization, Figure 5.2B). The increase in hepatocellular damage markers after mobilization did not relate to the extent of hepatic resection or the duration of mobilization (data not shown).

Liver mobilization results in hepatocyte apoptosis

Staining for M30 indicated that hepatocyte apoptosis tended to increase after mobilization (p=0.090) and returned to baseline after transection (Figure 5.3). There was a significant correlation between the duration of mobilization and the absolute increase in M30-positive cells (Pearson correlation=0.507, p=0.027).

Liver mobilization increases the number of hepatic immune cells

To study mobilization-mediated inflammation, inflammatory cells were identified in liver biopsies at 3 time points in 22 of the 25 included patients (88 per cent) by detection of MPO and CD68. Staining for MPO, a marker for neutrophils and macrophages, revealed a significant increase in absolute number of MPO-stained cells in liver tissue after mobi-

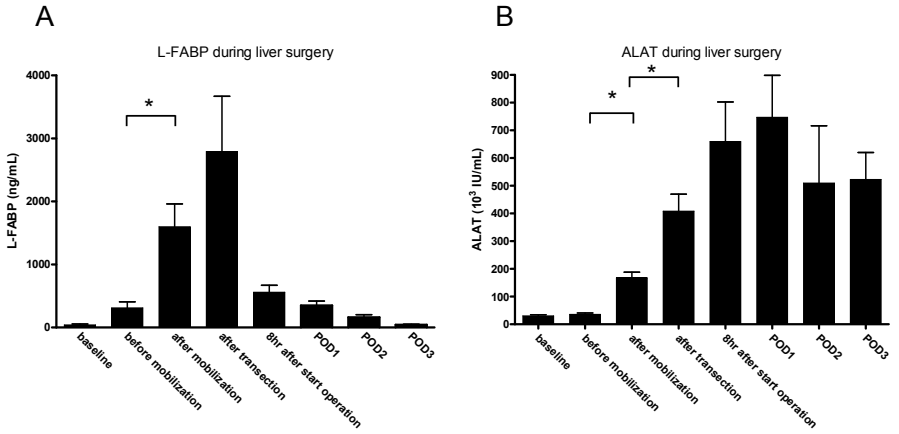

Figure 5.2A and 5.2B Course of hepatocellular damage markers L-FABP and ALAT during and after liver surgery.
(A) L-FABP levels increased significantly during mobilization of the liver. L-FABP levels peaked at the end of surgery and decreased thereafter (mean and standard error). **(B)** ALAT levels increased during mobilization of the liver and continued to increase significantly thereafter, until reaching their peak at the first postoperative day (mean and standard error).
Legend: L-FABP, liver fatty acid-binding protein; ALAT, alanine aminotransferase; POD, postoperative day; * indicates p<0.050.

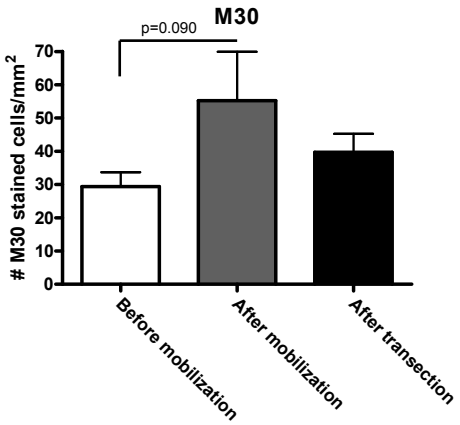

Figure 5.3 Apoptosis of hepatocytes during liver surgery as evidenced by M30-staining.
Liver mobilization resulted in a non-significant twofold increase in M30-stained cells (mean and standard error).

lization (p=0.001), which did not rise significantly during transection (p=0.080) (Figure 5.4A). There was a significant correlation between the absolute increase of MPO-positive cells after mobilization and the duration of mobilization (Pearson correlation=0.505, p=0.033).

Staining for CD68, a specific marker for macrophages, showed a small but significant increase in CD68-positive cells after mobilization (median score 2 [1-2.5] before versus 2.5 [2-3] after mobilization, p=0.002) (Figure 5.4B). These CD68-positive cells had a different morphology, characterized by enlargement and rounding after mobilization (median morphology score 1.5 [1-3] before versus 2 [1-3] after mobilization, p=0.003) (Figure 5.4C), suggesting that these cells represented monocytes that infiltrated the liver.

Liver mobilization induces hepatic expression of inflammatory genes

Enhanced expression of genes of pro-inflammatory cytokines, chemokines and adhesion molecules plays a significant role in promoting immune cell infiltration. In agreement with the histological findings, hepatic mRNA levels of IL-1β, IL-6 and IL-8 (Figure 5.5A-C) significantly increased after mobilization compared with baseline levels. Rise in expression ranged from 23-fold for IL-1β, 65-fold for IL-8 and 137-fold for IL-6. The expression of the chemokine IL-8 significantly correlated with the absolute increase in MPO-positive cells (Pearson correlation=0.516, p=0.049). After transection, the mRNA levels of IL-1β, IL-6 and IL-8 further increased.

The expression of the cell adhesion molecule ICAM-1, but not VCAM-1, increased significantly after mobilization (Figure 5.5D-E). The increase in ICAM-1 gene expression

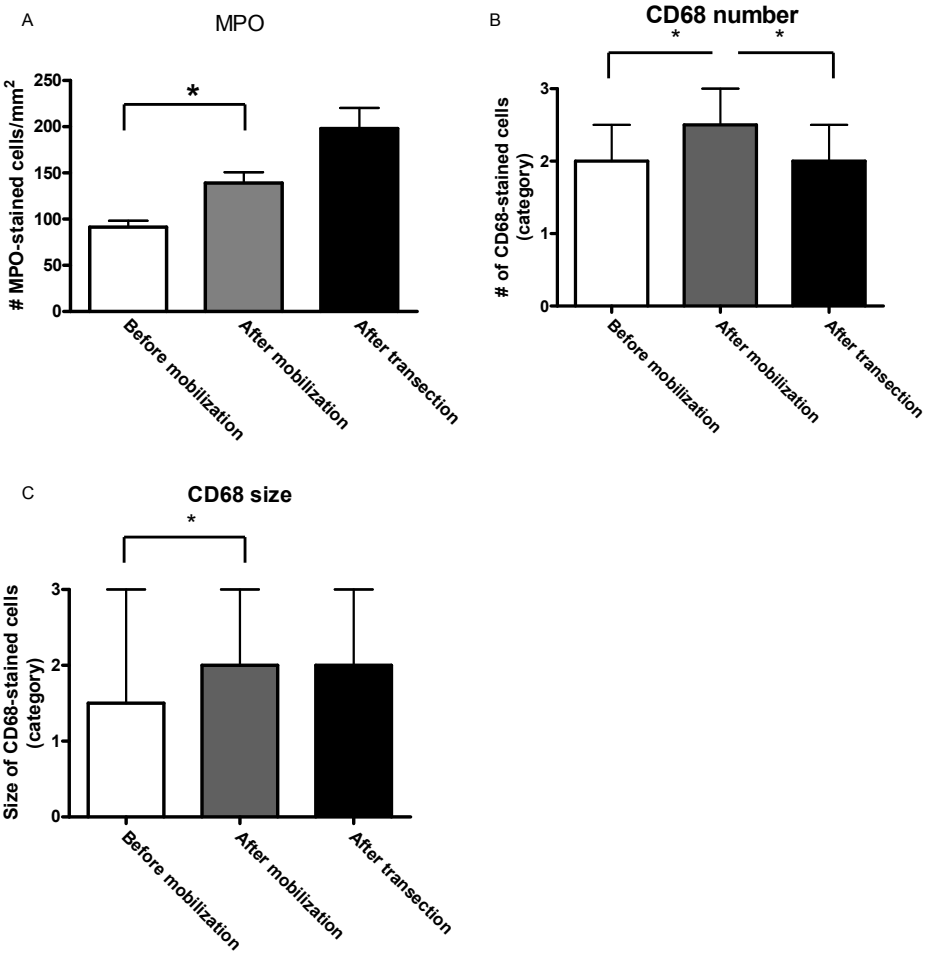

Figure 5.4A-C MPO and CD68-positive immune cells during liver surgery.
(A) Number of MPO-stained cells increased significantly during manipulation (mean and standard error). **(B)** CD68-staining revealed an increased number of CD68-positive cells in the liver after mobilization (median and range). **(C)** CD68-positive cells increased in size after mobilization of the liver (median and range). Legend: MPO, myeloperoxidase; CD68, cluster of differentiation 68; * indicates $p<0.050$.

tended to correlate with the absolute increase in MPO-positive cells (Pearson correlation=0.455, p=0.089).

DISCUSSION

The present study was designed to establish the association between liver mobilization, hepatocellular damage and hepatic inflammation during liver surgery in humans. Our data corroborate earlier observations that liver mobilization induces profound liver cell

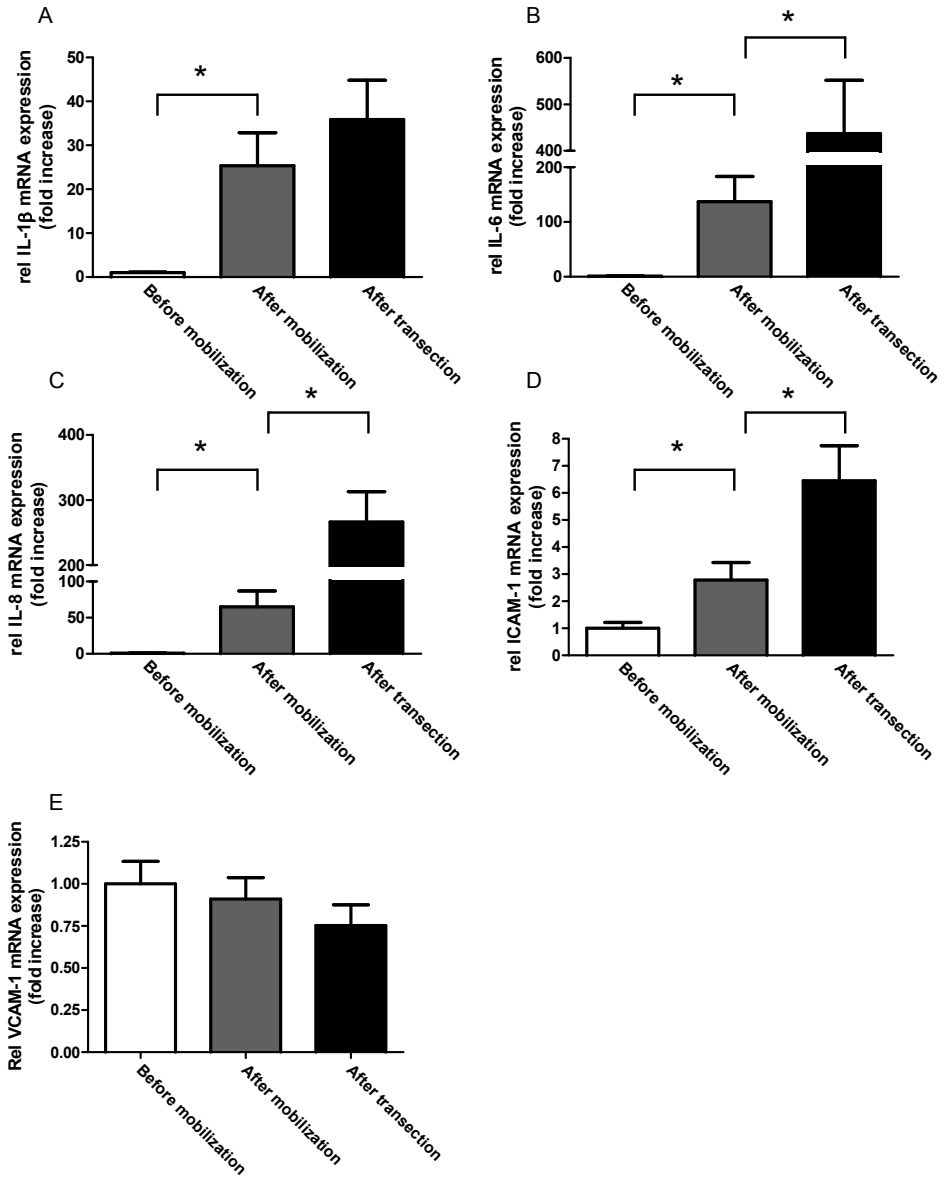

Figure 5.5A-E Relative hepatic gene expression of inflammatory cytokines and cell adhesion molecules during liver surgery.

(A-C) Relative expression of IL-1β, IL-6 and IL-8 significantly increased during liver surgery (mean and standard error). **(D-E)** Relative expression of ICAM-1 increased, whereas relative expression of VCAM-1 remained fairly constant (mean and standard error).

Legend: IL, interleukin; VCAM, vascular cell adhesion molecule; ICAM, intercellular adhesion molecule; * indicates $p<0.050$.

damage, as evidenced by an early and significant rise in the hepatocellular damage markers L-FABP and ALAT. In addition, we present novel, human data indicating that liver mobilization is associated with liver cell apoptosis and hepatic inflammation, as shown by an increase in MPO and CD68-positive inflammatory cells and upregulation of mRNA of pro-inflammatory cytokines in the liver. The extent of apoptosis and increase in inflammatory cells was significantly related to the duration of mobilization.

Manipulation of the liver immediately led to hepatocellular damage. Levels of the damage markers L-FABP and ALAT were significantly increased after mobilization and there was a trend towards increased hepatocyte apoptosis, evidenced by M30-positive hepatocytes. We previously showed that the increase in systemic plasma levels of L-FABP and ALAT after liver mobilization solely resulted from hepatic release and not from the hepatotoxic effects of anesthesia or surgical trauma of performing a laparotomy[3, 5]. Interestingly, the course of ALAT levels showed a different pattern compared to L-FABP levels. ALAT levels peaked on the first postoperative day, at the same time and in the same range as reported by other groups[19, 20], whereas L-FABP levels reached their maximum values at the end of surgery and rapidly decreased thereafter. This might be a reflection of the fact that L-FABP is a more direct and sensitive marker for the detection of liver cell damage as compared to ALAT, due to the small molecular mass and short half-life of L-FABP[16, 17, 21].

Inflammation has previously been recognized as an important element in the multifaceted process leading to manipulation-induced tissue injury in rodents[8, 22]. In man, however, the relation between liver manipulation, hepatocellular damage and hepatic inflammation was unknown. Here, we provide the first data in man showing that liver mobilization is accompanied by hepatocyte apoptosis and influx of inflammatory cells in hepatic tissue. Additional CD68-staining identified involvement of monocytes and macrophages. It has previously been shown that macrophages such as Kupffer cells are able to express MPO, which might reflect their pro-inflammatory status[18, 23]. The present findings of enhanced hepatic expression of the typical pro-inflammatory macrophage markers IL-1β and IL-6 are in line with this hypothesis.

It might be clinically relevant to prevent mobilization-induced liver cell damage, hepatocyte apoptosis and inflammation by either employment of alternative surgical techniques or modulation of inflammation. With respect to the latter, it remains unclear whether the presence and activation of inflammatory cells secondary to liver manipulation is beneficial or not. In general, unrestrained activation of inflammatory cells following trauma is believed to exacerbate damage, although initially intended to maintain homeostasis[24, 25]. As human evidence on the clinical consequences of mobilization-induced liver inflammation is lacking to date, assumptions are solely based on results of animal studies. In animal models of liver manipulation-induced hepatocellular damage, the administration of gadolinium chloride, a Kupffer cell toxicant, or glycine, which prevents

Kupffer cell activation, led to decreased hepatocellular damage and improved survival after liver transplantation[8, 26]. In other areas of research, modulation of inflammation secondary to manipulation has proven to be beneficial in terms of clinical outcomes in animals as well as humans[27-29]. Intervention studies with anti-inflammatory drugs, aiming at a modulation of monocyte influx or macrophage activity, may be performed to elucidate whether a dampened inflammatory response would lead to less tissue injury and, more importantly, improved clinical outcome in patients undergoing liver surgery.

Alternative surgical techniques that require less manipulation of the liver, are already available, such as laparoscopic liver resection or liver resection using the anterior approach[30, 31]. Laparoscopy might be advantageous, although several reports show an unfavorable effect of the pneumoperitoneum on hepatic microcirculation[32, 33]. This is undesirable as animal study data suggest that microcirculatory failure mediates manipulation-induced liver cell damage[8]. Liver resection using the anterior approach involves initial completion of parenchymal transection without mobilization of the right hemi-liver[30, 34]. Advantages include minimal interruption of hepatic circulation during surgery, improved liver function and reduced risk of spilling viable cancer cells into the circulation, at the cost of an enhanced risk of bleeding[30]. Indeed, Liu and coworkers showed reduced cell-free circulating albumin-mRNA levels, as a marker of circulating liver cells, and a lower incidence of PLF in patients undergoing liver resection using the anterior approach compared with the conventional approach[7]. Comparison of hepatocellular damage, hepatic inflammation and clinical outcomes between patients undergoing liver surgery using the conventional approach versus the anterior approach is warranted.

The trigger for immune activation in mobilization-induced liver damage in man remains to be identified. Oxidative stress-related danger signals, resulting from microcirculatory failure, might well be involved as triggers of local inflammation[11]. Livers with reduced anti-oxidant capacity and pre-existent microvascular damage, such as livers suffering from chemotherapy-associated hepatotoxicity, may therefore be at additional risk[35, 36]. Moreover, the relation between mobilization-induced hepatocellular damage, hepatic inflammation and clinical outcome remains to be established in a trial using larger patient groups[37].

Taken together, the results of the present study provide evidence of an association between liver mobilization, hepatocellular damage and hepatic inflammation in man, in line with previous results from animal studies. They form the basis for the development of novel therapies to prevent mobilization-induced damage early during liver surgery, such as the administration of immune-modulating drugs or adoption of alternative surgical techniques.

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Chapter 6

Randomized controlled trial analyzing the effect of 15 or 30 min intermittent Pringle maneuver on hepatocellular damage during liver surgery

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ABSTRACT

Background

Aminotransferases are commonly used to determine the optimal duration of ischemic intervals during an intermittent Pringle maneuver (IPM). However, they might not be responsive enough to detect small differences in hepatocellular damage. Liver fatty acid-binding protein (L-FABP) has been suggested as a more sensitive marker. This randomized trial aimed to compare hepatocellular injury reflected by L-FABP in patients undergoing liver resection with IPM using 15 or 30 min ischemic intervals.

Methods

Twenty patients undergoing liver surgery were randomly assigned to IPM with 15 (15IPM) or 30 (30IPM) min ischemic intervals. Ten patients not requiring IPM (noIPM) served as controls. Primary endpoint was hepatocellular injury during liver surgery reflected by systemic L-FABP plasma levels. Between group comparisons were performed using area under the curve analysis and repeated measures two-way ANOVA.

Results

The IPM groups had similar characteristics. Aminotransferases did not differ significantly between 15IPM and 30IPM at any time point. L-FABP levels rose up to 1853 ± 708 ng/mL in the 15IPM and 3662 ± 1355 ng/mL in the 30IPM group after finishing liver transection and decreased rapidly thereafter. There were no significant differences between 15IPM and 30IPM in cumulative L-FABP level ($p=0.378$) or L-FABP level at any time point ($p=0.149$). Blood loss, remnant liver function and morbidity were comparable.

Conclusion

IPM with 15 or 30 min ischemic intervals induced similar hepatocellular injury measured by the sensitive marker L-FABP. The present study confirms the results of earlier trials, suggesting that IPM with 30 min ischemic intervals may be used.

INTRODUCTION

Hepatic inflow occlusion (Pringle maneuver) is used to minimize blood loss during liver surgery, as excessive intra-operative blood loss and red blood cell transfusions adversely affect short- and long-term outcomes[1-3]. Different clamping techniques can be applied, such as partial or complete hepatic inflow occlusion using either continuous or intermittent pedicle clamping[4]. Generally, intermittent clamping is regarded superior to continuous clamping as it results in a better preserved remnant liver function[5]. The optimal duration of the ischemic intervals during an intermittent Pringle maneuver (IPM) is unknown and depends on the balance between ischemia-induced hepatocellular damage and blood loss. As each period of reperfusion is associated with increased blood loss[5-7], prolonged ischemic intervals might be preferable. Indeed, two randomized trials showed that complete IPM using 30 min ischemic intervals resulted in similar remnant liver function and hepatocellular damage compared to IPM using 15 min ischemic intervals, while intra-operative blood loss was lower in the 30 min ischemic interval groups[6, 8].

Hepatocellular damage after pedicle clamping is commonly evaluated using alanine and aspartate aminotransferase (ALAT and ASAT) levels on consecutive postoperative days[7-9]. However, it remains uncertain if the assay of aminotransferases is sufficiently sensitive to detect small differences in hepatocellular injury[9]. Due to the relatively large molecular mass (96 kDa) and long half-life of aminotransferases, their plasma levels react slowly to acute tissue damage. In addition, aminotransferase levels on the first postoperative days may not only reflect ischemia-induced hepatocellular damage, but also effects of postoperative care such as drug-induced hepatotoxicity or blood transfusions. Furthermore, aminotransferases, and especially ASAT, have modest organ specificity[10]. More accurate markers for the detection and monitoring of hepatocellular injury in man are available. One of these markers is liver fatty acid-binding protein (L-FABP)[11, 12]. L-FABP is a cytosolic protein that is abundantly present in liver tissue. Its biological function involves facilitation of intracellular fatty acid transport and participation in lipid metabolism[12]. After hepatocyte damage, it diffuses quickly into the circulation because of its small mass (≈ 13-14 kDa). Circulating L-FABP, released from damaged cells, is cleared by the kidneys with a half-life of 11 min and as a result, plasma levels rapidly return to normal[13].

The present randomized controlled trial aimed to assess the effect of complete IPM using either 15 or 30 min ischemic intervals on hepatocellular injury reflected by L-FABP as opposed to the less sensitive damage markers ALAT and ASAT.

METHODS

Experimental design

Consecutive patients scheduled to undergo liver surgery at Maastricht University Medical Centre and willing to participate were enrolled in this prospective randomized controlled trial. Exclusion criteria were (1) presence of liver cirrhosis confirmed by biopsy, (2) concomitant extra-hepatic procedures or bilio-enteric anastomosis, (3) steroid hormone medication, (4) renal dysfunction defined as serum creatinine above 137 µmol/L in men and above 104 µmol/L in women[14], and (5) laparoscopic liver resection.

Immediately after the surgeon decided complete IPM would be required during liver transection, patients were randomized in a 1:1 ratio to receive either IPM with 15 min ischemic intervals (15IPM) or 30 min ischemic intervals (30IPM), both followed by 5 min reperfusion. Randomization was performed in the operating theatre by a researcher using numbered, sealed, opaque envelopes. An independent researcher generated the randomization sequence. Patients were blinded to the allocated intervention. Patients who did not require IPM (noIPM) served as controls.

The study protocol was approved by the Medical Ethics Committee of Maastricht University Medical Centre and registered at ClinicalTrials.gov NCT01099475. The manuscript complies with the updated CONSORT guidelines[15]. Informed consent was obtained prior to surgery.

Surgical procedure

Patients were anaesthetized using propofol and isoflurane. They routinely had an epidural catheter, urinary catheter, two peripheral venous catheters and indwelling catheters in a jugular vein and radial artery. Body temperature was maintained using a Bair Hugger system (Arizant Healthcare Inc., Eden Prairie, MN, USA). The surgical procedure was performed using a subcostal bilateral incision and Olivier retractors to improve exposure[16]. After dissection of the teres hepatis ligament, the liver was mobilized. Thereafter, an intra-operative ultrasound was performed to define the position of the tumour in relation to vascular and biliary structures. As IPM was not routinely applied, a patient was randomized for 15IPM or 30IPM only after the surgeon had decided a complete Pringle maneuver would be required. During 15IPM or 30IPM, the complete portal triad was clamped using a rubber sling. The time of inflow occlusion was adapted to the need according to the randomization protocol. Occasionally, the left or right pedicle was ligated after protocolled IPM. Five min reperfusion intervals were applied during which transection was stopped and cut surfaces were gently compressed to ensure hemostasis. A Cavitron Ultrasonic Surgical Aspirator (CUSA system 200 macrodissector, Cavitron Surgical Systems, Stamford, CT, USA) and Argon beam coagulation (Force GSU System, Valleylab, Boulder, CO, USA) were used for liver transection. A stapler device or clamps

were used for transection of the hepatic veins. Central venous pressure was maintained below 5 mmHg during transection to reduce venous back-bleeding. After surgery, the weight of the resection specimen was recorded. Perioperative care was protocolled, as described earlier[17].

Blood sampling

Arterial blood samples were drawn from the radial artery catheter according to a fixed protocol (Figure 6.1). Before and after parenchymal transection, blood was sampled from the portal vein and one of the hepatic veins from the non-tumorous side of the liver by direct puncture. Blood samples were transferred to prechilled EDTA tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ, USA). The tubes were centrifuged at 4°C at 3500 x g for 15 min to separate plasma and stored in aliquots at –80°C till batch analysis.

Outcome measures

The primary endpoint was hepatocellular damage reflected by systemic L-FABP plasma levels during and after liver surgery. Secondary endpoints were hepatocellular damage reflected by ALAT and ASAT, hepatic function reflected by total bilirubin level and prothrombin time, cumulative ischemia and reperfusion time, duration of operation, amount of intra-operative blood loss, blood loss per gram resected liver weight, need for red blood cell transfusion during surgery or within 48hr after surgery, and morbidity and 30-day mortality. Resections were divided into major (3 or more segments) or minor (less than 3 segments or non-anatomical resections)[18]. The clinical course of the participants was studied prospectively. Postoperative complications were graded according to the Clavien-Dindo score[19]. Grades 1 and 2 were regarded as minor and grades 3, 4 and 5 as major morbidity. Post-resectional liver failure was defined according to the 50-50

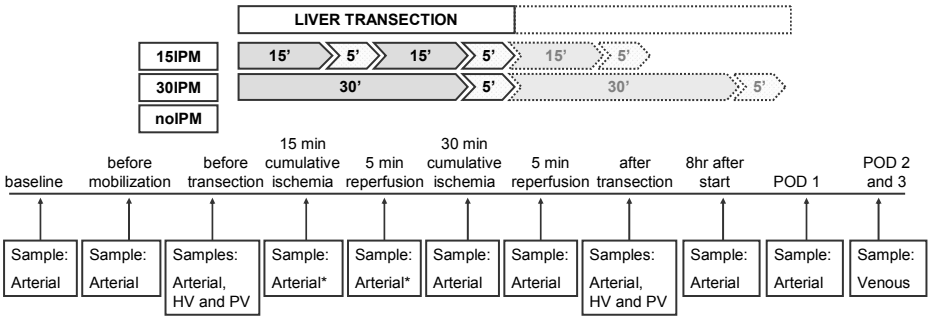


Figure 6.1 Study and sampling protocol.
Legend: 15IPM, 15 min intermittent Pringle maneuver; 30IPM, 30 min intermittent Pringle maneuver; noIPM, no intermittent Pringle maneuver; HV, hepatic vein; PV, portal vein; POD, postoperative day; * only in 15IPM and noIPM group.

criteria on postoperative day 5[20]. Bile leakage was considered in case of (1) leakage of any quantity of bile via the abdominal wound or drain at least 48hr postoperatively; (2) intra-abdominal collection of bile at the time of re-operation or percutaneous drainage; or (3) cholangiographic evidence of contrast leakage. Intra-abdominal abscess was present if there was (1) leakage of any quantity of purulent fluid via the abdominal drain or (2) intra-abdominal collection of pus at the time of re-operation or percutaneous drainage, both combined with a positive microbiological culture.

Determination of hepatocellular damage

L-FABP was used as a marker of liver tissue damage. L-FABP plasma levels were determined using a commercially available ELISA (Hycult Biotechnology, Uden, the Netherlands). According to the manufacturer's manual, L-FABP plasma levels in healthy individuals were approximately 12 ng/mL. ALAT and ASAT levels were determined by the clinical chemistry laboratory of Maastricht University Medical Centre. The upper limit of normal was 35 IU/L for ALAT and 30 IU/L for ASAT.

Source of L-FABP before and after IPM

L-FABP is highly expressed in the liver, intestine and kidneys[12]. As a result, systemic L-FABP plasma levels during liver surgery can reflect either hepatic, intestinal or renal damage[21]. The source of systemic L-FABP plasma levels was therefore determined by sampling blood from the portal and hepatic vein simultaneously with an arterial sample and then by calculation of arteriovenous differences (ΔAV) and net organ fluxes (F; flow x ΔAV) across the splanchnic area, portal drained viscera (PDV) and liver. Plasma flow was measured in a previous similar series of patients and amounted to 320 ± 42 mL/min in the portal vein and 110 ± 23 mL/min in the hepatic artery[16]. Splanchnic flow was calculated as portal vein plus hepatic artery plasma flow. Fluxes were calculated as F_{PDV}= portal plasma flow * ([portal vein] – [artery]), $F_{splanchnic}$= splanchnic plasma flow * ([hepatic vein] – [artery]) and F_{liver}= $F_{splanchnic}$ – F_{PDV} and corrected for body weight. Positive fluxes indicate release, whilst negative fluxes indicate uptake.

Liver histology

The presence of underlying disease in the non-tumorous liver was assessed by an experienced HPB pathologist in the resection specimen using haematoxylin and eosin staining. The presence of more than 30 per cent hepatic steatosis, grades 1 to 3 fibrosis and nodular regenerative hyperplasia was evaluated in liver tissue distant from the tumour.

Statistical analysis

Based on previous observations, mean L-FABP plasma level after transection using IPM with 15 min ischemic and 5 min reperfusion intervals was 775 ± 210 ng/mL[13]. The

present study was powered to detect a 100 per cent relative difference in L-FABP levels between the groups with 15 or 30 min ischemic intervals, favouring the 15 min group. The 100 per cent difference was chosen because it was regarded clinically relevant and precluded large influences of analytical variation. Assuming $\alpha = 0.05$ and $\beta = 0.2$ with two-sided testing, 10 patients would be required in each study arm.

Continuous data are expressed as mean and standard error if normally distributed or median and range if not normally distributed. Dichotomous data are presented as absolute numbers.

Differences in baseline, operative characteristics and postoperative outcomes between the 15IPM and 30IPM group were tested using a Mann-Whitney U test. Dichotomous data were compared using Pearson's chi-square test. Continuous variables with repeated measurements were summarized as area under the curve (AUC) from baseline to postoperative day 3[22]. AUCs of the 15IPM and 30IPM group were compared using the Student's t-test. Subsequently, the two IPM groups were compared with the noIPM group. As a third step, differences in hepatocellular damage markers at specific time points within and between the 15IPM and 30IPM group were analyzed using the repeated measures two-way ANOVA with Bonferroni correction if the p-value was below 0.050. L-FABP fluxes before and after liver transection were tested using a one-sample t-test with a theoretical mean of zero. A p-value below 0.050 was considered statistically significant.

RESULTS

Participant flow

One-hundred and two patients were assessed between October 2007 and July 2009, of whom 60 were potentially eligible for inclusion. A number of patients who did meet the inclusion criteria were not eligible because of a simultaneous randomized trial on enhanced recovery after surgery. Out of these 60 patients, 30 patients were included in the present study consisting of 10 patients in the 15IPM group, 10 patients in the 30IPM group and 10 patients in the control group (Figure 6.2). All patients received the allocated intervention. Of the 20 IPM patients, three needed more than two-times 15 min and five more than one-time 30 min intermittent inflow occlusion. In the noIPM group, five patients underwent a liver resection without any form of inflow occlusion and five with pedicle ligation to either the left or right side. No unplanned complete hepatic inflow occlusion was required in this group. No adverse events related to the intervention were encountered.

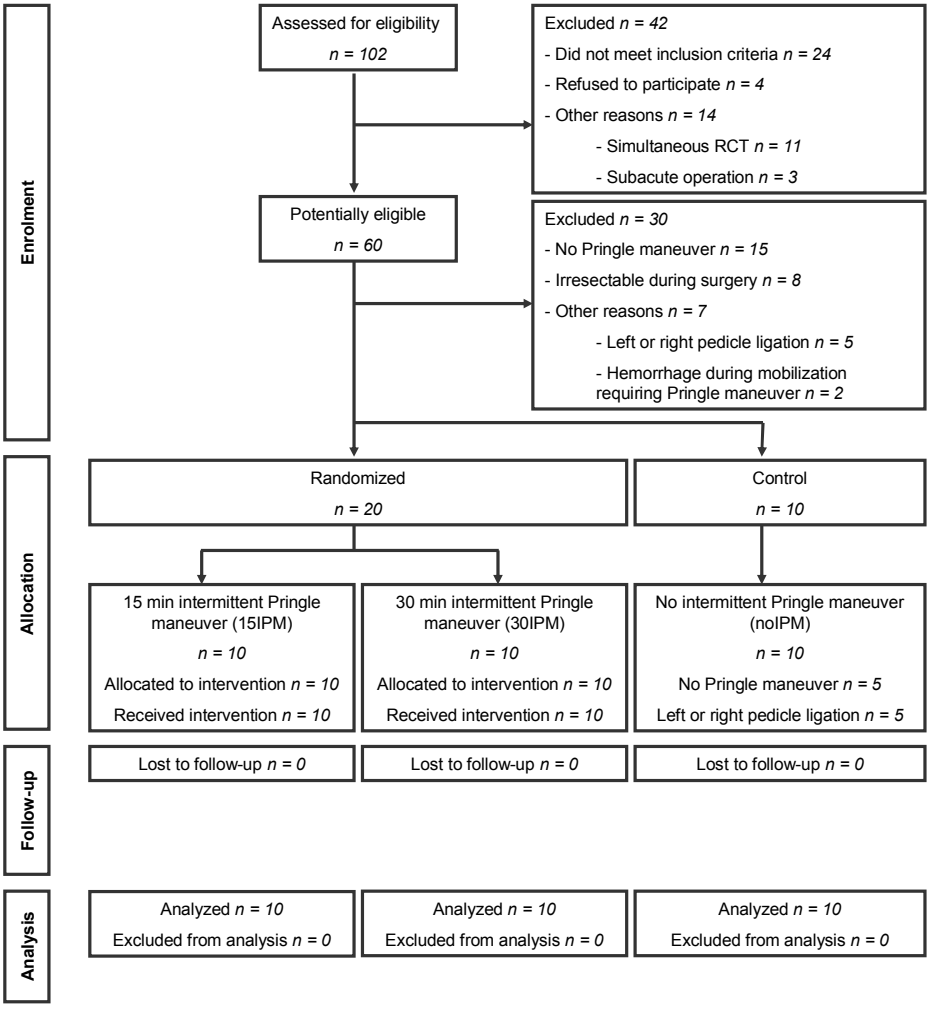


Figure 6.2 CONSORT diagram of patient selection.
Legend: *N*, number; RCT, randomized controlled trial; 15IPM, 15 min intermittent Pringle maneuver; 30IPM, 30 min intermittent Pringle maneuver; noIPM, no intermittent Pringle maneuver.

Patient characteristics

Table 6.1 shows the patients' demographics and operative characteristics. The indications for liver resection were colorectal liver metastases in 26 patients, primary liver cancer in non-cirrhotic liver in two patients and hepatic adenoma and liver metastases from a carcinoid tumour in one patient each. There were no differences in baseline or operative characteristics between the 15IPM and 30IPM group, except for baseline total bilirubin level, which was significantly higher in the 15IPM group (Table 6.1). The cumulative ischemia time was 34 min (28-105 min) in the 15IPM and 51 min (30-65 min) in the

Table 6.1 Characteristics of patients included in the present randomized controlled trial.

	Control group	Intervention groups		
	noIPM (*n* = 10)	15IPM (*n* = 10)	30IPM (*n* = 10)	p-value*
Baseline characteristics				
Age (years)	60 (41-70)	64 (48-80)	62 (30-77)	0.631
Sex (male)	7	6	6	1.000
BMI (kg/m^2)	24 (22-31)	24 (20-31)	26 (20-33)	0.684
ASA classification				0.443
ASA I	2	0	2	
ASA II	8	8	5	
ASA III	0	2	3	
Preoperative laboratory tests				
Bilirubin (total) (µmol/L)	15 (7-37)	14 (11-17)	10 (8-16)	0.046
Prothrombin time (sec)	11 (10-11)	11 (10-14)	10 (10-11)	0.348
Creatinine (µmol/L)	90 (54-103)	89 (59-128)	83 (68-136)	0.617
Indication for resection				1.000
Benign lesions	0	0	1	
Malignant lesions	10	10	9	
Operative characteristics				
Type of resection				0.650
Major hepatectomy	3	5	3	
Minor hepatectomy	7	5	7	
Number of resected segments	2 (1-5)	2 (1-4)	2 (1-5)	0.964
Resected liver weight (grams)	224 (51-1065)	317 (95-934)	266 (41-1140)	0.764
Duration of surgery (min)	195 (150-345)	218 (120-315)	235 (165-330)	0.323
Mobilization time (min)	48 (20-85)	55 (40-130)	55 (25-105)	0.762
Transection time (min)	78 (55-160)	75 (40-155)	110 (31-146)	0.315
Cumulative ischemia time (min)	0	34 (28-105)	51 (30-65)	0.380
Cumulative release time (min)	0	6 (4-41)	5 (0-14)	0.113
Histology of non-tumorous liver				1.000
Normal	7	6	6	
Steatosis > 30 per cent	1	1	2	
NRH	2	1	1	
Fibrosis grade 1-3	0	2	1	
Intra-operative blood loss (mL)	500 (200-1200)	575 (100-2300)	450 (250-1000)	0.915

Numbers indicate median (range) or absolute number; N, number; noIPM, no intermittent Pringle maneuver; 15IPM, 15 min intermittent Pringle maneuver; 30IPM, 30 min intermittent Pringle maneuver; BMI, body mass index; ASA, American Society of Anaesthesiologists; NRH, nodular regenerative hyperplasia; * p-value represents comparison between 15IPM and 30IPM group.

30IPM group (p=0.380). In the 15IPM group, median blood loss was 575 mL (100-2300 mL) compared with 450 mL (250-1000 mL) in the 30IPM group (p=0.915). Blood loss per gram resected liver weight was 1.3 mL/gram (0.3-19.0 mL/gram) in the 15IPM and 1.4 mL/gram (0.3-9.8 mL/gram) in the 30IPM group (p=0.887). Also transection times were comparable.

Hepatocellular damage

The degree of hepatocellular damage reflected by L-FABP, ALAT and ASAT in the 15IPM, 30IPM and noIPM group is depicted in Figures 6.3A, 6.3B and 6.3C. Exact concentrations at different time points are depicted in Appendix 10. Already before parenchymal transection, systemic L-FABP levels were significantly increased compared with baseline levels in all groups. L-FABP levels reached their peak just after transection and rapidly returned to baseline levels after surgery.

Systemic ALAT and ASAT levels showed a different pattern. Aminotransferase levels increased significantly during mobilization of the liver and rose further after transection. ALAT and ASAT peaked 8hr after the start of surgery and remained elevated during the first postoperative days. The mean AUC of L-FABP from baseline up until 3 days after surgery was 9097 ± 1781 in the 15IPM and 11 688 ± 2247 in the 30IPM group (p=0.378). In the noIPM group, the mean AUC was 6374 ± 760, which was significantly less than the mean AUC of the IPM groups (p=0.019). For ALAT and ASAT, the mean AUCs were 3196 ± 797 and 3147 ± 713 in the 15IPM and 3609 ± 812 and 3800 ± 799 in the 30IPM group, respectively (p=0.721 and p=0.550). The noIPM group showed an AUC for ALAT of 2075 ± 332 and for ASAT of 2129 ± 276 (p=0.049 and p=0.094 versus the IPM groups).

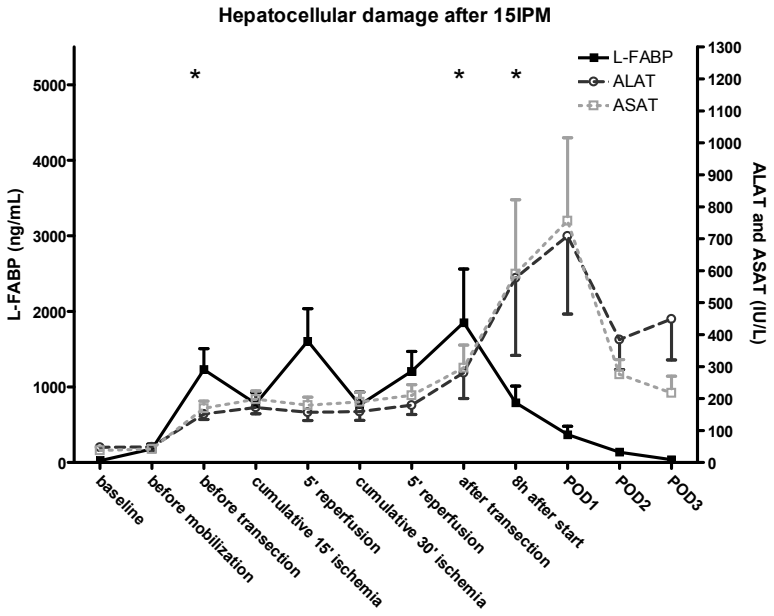
Furthermore, there was no significant difference between the 15IPM and 30IPM group in systemic L-FABP levels at any time point (overall p-value two-way repeated measures ANOVA p=0.149). The same holds for ALAT and ASAT levels (overall p-value two-way repeated measures ANOVA p=0.149 and p=0.116, respectively).

Source of L-FABP before and after IPM

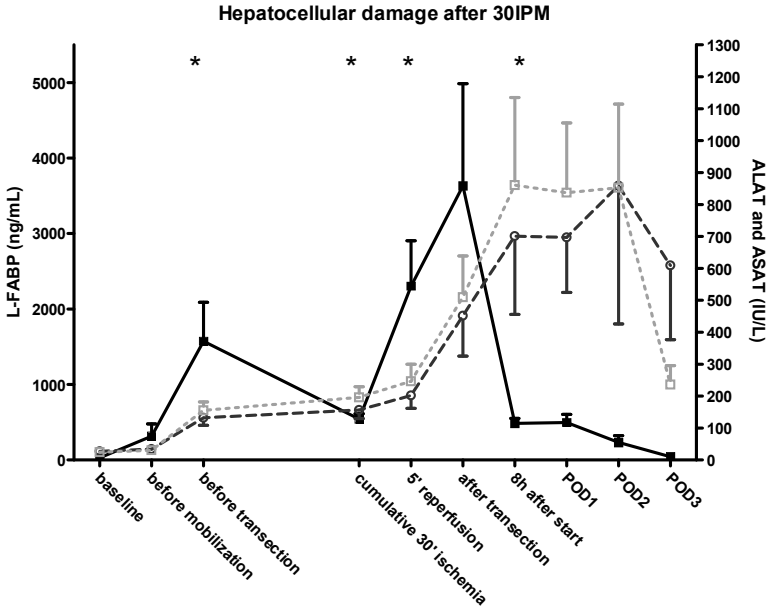
Before liver transection, there was a significant release of L-FABP from the liver in the 15IPM and noIPM group (Figure 6.4A), indicating that mobilization of the liver induced liver cell damage.

After transection, there was a remarkable difference between L-FABP flux in the groups with 15IPM and 30IPM versus the noIPM group, suggesting the induction of ischemic hepatocellular damage by the Pringle maneuver. There was no evidence for intestinal injury secondary to splanchnic vascular congestion during IPM as the PDV did not significantly release L-FABP after transection with IPM using either 15 or 30 min ischemic intervals (Figure 6.4B).

A


Hepatocellular damage after 15IPM
L-FABP
ALAT
ASAT
L-FABP (ng/mL)
ALAT and ASAT (IU/L)
baseline
before mobilization
before transection
cumulative 15' ischemia
5' reperfusion
cumulative 30' ischemia
5' reperfusion
after transection
8h after start
POD1
POD2
POD3

B


Hepatocellular damage after 30IPM
L-FABP (ng/mL)
ALAT and ASAT (IU/L)
baseline
before mobilization
before transection
cumulative 30' ischemia
5' reperfusion
after transection
8h after start
POD1
POD2
POD3

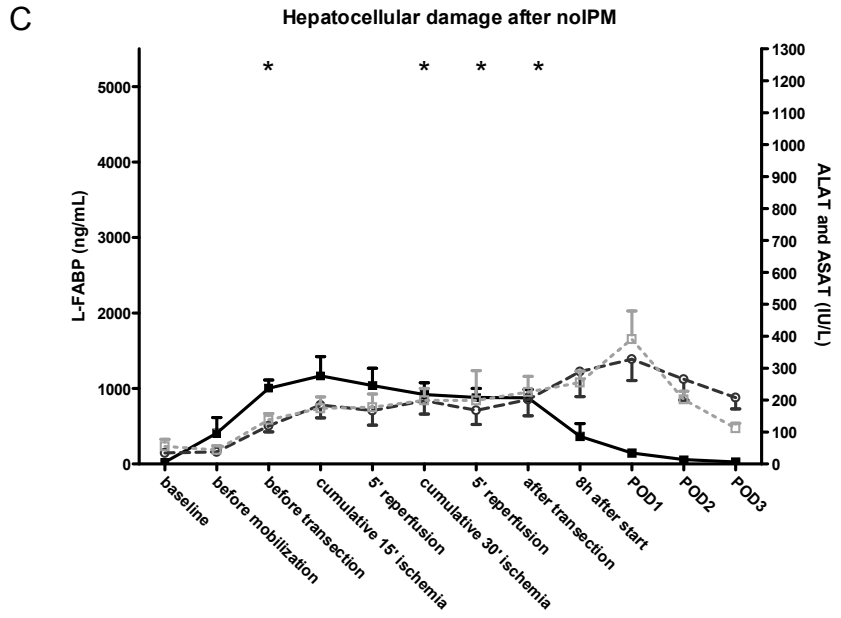


Figure 6.3A-C Course of L-FABP (continuous black line), ALAT (interrupted black line), and ASAT (dotted grey line) in the 15IPM **(A)**, 30IPM **(B)**, and noIPM group **(C)**.
Legend: Data are presented as mean and standard error. Data after cumulative 15' ischemia and 5' reperfusion lack in the 30IPM group; L-FABP, liver fatty acid-binding protein; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; 15IPM, 15 min intermittent Pringle maneuver; 30IPM, 30 min intermittent Pringle maneuver; noIPM, no intermittent Pringle maneuver; * $p<0.050$ for L-FABP versus baseline calculated with repeated measures two-way ANOVA with post-hoc paired sample t-test.

Postoperative course

The postoperative course is depicted in Table 6.2. Seventeen patients experienced postoperative complications (57 per cent), of whom 8 had only minor and nine a combination of minor and major complications. Two patients died within 30 days after liver surgery, accounting for a 30-day mortality rate of 7 per cent. The reasons of death were sepsis with multi-organ failure and post-resectional liver failure in 1 patient each. The 30-day mortality in the total cohort of patients (n = 102) who had undergone liver resection during the inclusion period of the present study was 4 per cent. The complications in the two IPM groups were comparable. Four patients in the 15IPM group and two in the 30IPM group required a red blood cell transfusion (p=0.628).

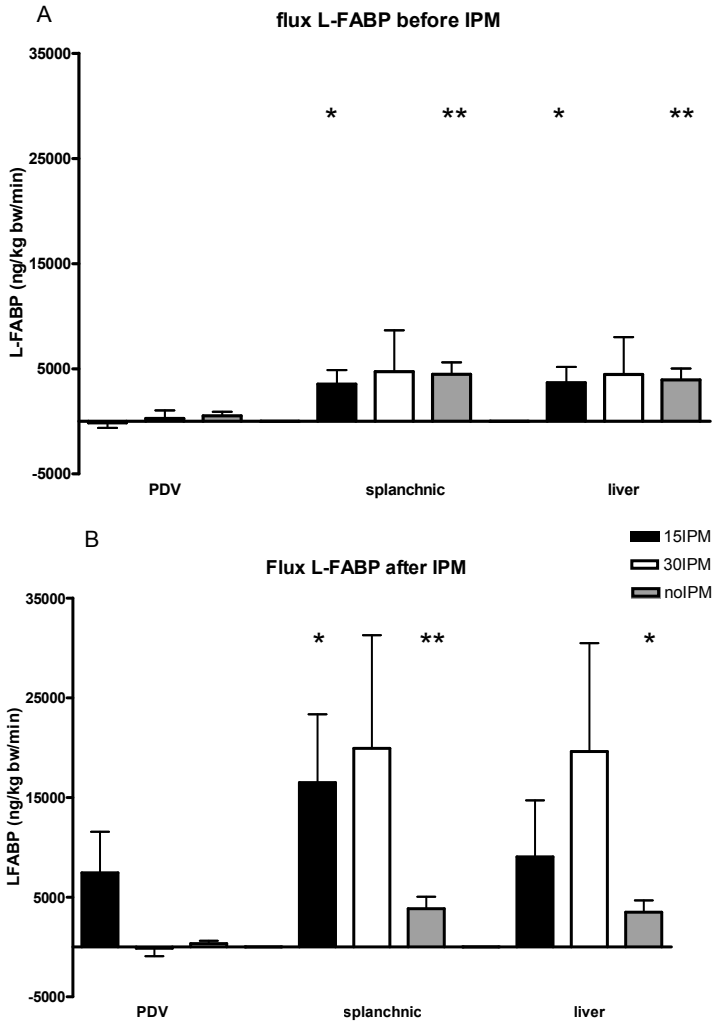


Figure 6.4A and 6.4B Fluxes of L-FABP before and after liver transection with and without intermittent Pringle maneuver.

Legend: Data are presented as mean and standard error; PDV, portal drained viscera; L-FABP, liver fatty acid-binding protein; IPM, intermittent Pringle maneuver; * $p<0.050$ and ** $p<0.010$.

DISCUSSION

The present randomized controlled trial was designed to compare the effect of intermittent hepatic inflow occlusion using either 15 or 30 min ischemic intervals on hepatocellular damage during liver surgery in man. In addition to the commonly used aminotransferases, the more sensitive marker L-FABP was studied as a read out for liver injury.

Table 6.2 Postoperative course of patients undergoing liver surgery with or without intermittent Pringle maneuver.

	Control group	Intervention groups		
	noIPM (*n* = 10)	15IPM (*n* = 10)	30IPM (*n* = 10)	p-value*
Postoperative liver function				
Peak bilirubin (total) (μmol/L)	23 (16-101)	31 (22-194)	37 (14-84)	0.670
Peak prothrombin time (sec)	13 (11-21)	14 (11-62)	15 (11-21)	0.324
Minor complications#				
Pneumonia	1	3	1	0.582
Pleural effusion	1	2	1	1.000
Wound infection	1	1	0	1.000
Ileus	1	1	1	1.000
Red blood cell transfusion	1	4	2	0.628
Units	1 (n.a.)	4 (2-7)	2 (1-2)	0.267
Major complications#				
Intra-abdominal abscess	0	3	2	1.000
Intra-abdominal hemorrhage	0	0	1	1.000
Bile leakage	0	4	1	0.303
Ascites	0	1	0	1.000
Sepsis	0	1	2	1.000
Post-resectional liver failure	1	2	1	1.000
30-day mortality	0	1	1	1.000
Postoperative stay				
Length of hospital stay (days)	8 (5-10)	11 (5-53)	8 (5-119)	0.955

Numbers indicate median (range) or absolute number; N, number; noIPM, no intermittent Pringle maneuver; 15IPM, 15 min intermittent Pringle maneuver; 30IPM, 30 min intermittent Pringle maneuver; n.a., not applicable; # graded according to the Clavien-Dindo classification[19]; * p-value represents comparison between 15IPM and 30IPM group.

We provide novel data that show no difference in hepatocellular damage reflected by L-FABP between patients undergoing liver resection with complete IPM using either 15 or 30 min ischemic intervals. These results corroborate results of earlier trials which used less sensitive markers for liver tissue damage. Intra-operative blood loss, red cell blood transfusions and remnant liver function were comparable. Moreover, complete IPM did not induce intestinal injury secondary to portovenous stasis.

Hepatic inflow occlusion is frequently applied during liver transection to prevent excessive blood loss[4, 23]. A drawback of the Pringle maneuver is the induction of ischemic hepatocellular injury resulting in postoperative morbidity[24, 25]. In order to limit this injury, techniques such as ischemic preconditioning and intermittent pedicle clamping are effective[5, 7]. However, several trials suggested that IPM using 15 min ischemic intervals was associated with significantly higher blood loss during transection compared with

continuous clamping, resulting from extensive bleeding during the 5 min reperfusion periods[5, 7]. Theoretically, ischemic intervals of 30 min are preferable as this time period allows the surgeon to control vessels at the cut surface of the liver meticulously whereas the liver can tolerate cumulative ischemia up to 120 min[26]. Moreover, short ischemic intervals lead to repetitive reperfusion episodes, potentially resulting in more extensive hepatocellular damage. Two recent randomized trials did show that remnant liver function and hepatocellular damage after IPM using 15 or 30 min ischemic intervals were comparable, whereas blood loss was lower after 30IPM[6, 8]. In these studies, however, bilirubin or aminotransferase levels on different postoperative days were used as a read out for liver function and damage while it is questionable whether these levels are sufficiently sensitive to pick up slight differences between the two ischemic intervals[10]. Our trial confirms that hepatocellular damage, measured by the highly sensitive damage marker L-FABP, was similar between complete IPM with 15 or 30 min ischemic intervals. Also remnant liver function was comparable. However, no IPM resulted in significantly less hepatocellular damage in the control group of patients with similar characteristics. As a recent meta-analysis showed no definite advantage of any form of portal triad clamping in terms of postoperative outcomes, IPM should be preserved for individual patients undergoing complex resections with expected high blood loss[27]. If a Pringle maneuver is necessary, 30 min ischemic intervals should be used as this led to decreased blood loss and a higher transection speed in previous studies with larger patient numbers[6, 8].

Another concern of the Pringle maneuver is the induction of intestinal damage secondary to portovenous stasis[28]. Theoretically, intestinal injury after hepatic inflow occlusion might result in intestinal barrier dysfunction and bacterial translocation. The clinical relevance of clamping-induced intestinal injury in man is under debate[28, 29]. Kim and colleagues described an increase in portovenous interleukin-6 levels compared with arterial levels at the end of continuous inflow occlusion, suggesting interleukin-6 production in the mesenteric area[28]. A small study by Huguet's group showed that, even though bacterial translocation to mesenteric lymph nodes occurred during liver resection under continuous inflow occlusion, no correlation between positive lymph nodes and postoperative infectious complications was present[29]. Our data provide clear evidence that complete IPM using either 15 or 30 min inflow occlusion induced no significant intestinal damage, evidenced by minimal L-FABP fluxes over the portal drained viscera. L-FABP is abundantly present in intestinal villi and intestinal damage typically results in a rapid release of L-FABP[21]. Therefore, L-FABP plasma concentration is regarded a reliable marker for the detection of intestinal injury. The exact relation between L-FABP plasma level and gut barrier function has, to the best of our knowledge, not yet been elucidated. As a result, the effect of IPM with either 15 or 30 min inflow occlusion on intestinal barrier function remains to be established. By measuring fluxes,

an exclusive hepatic origin of systemic L-FABP plasma levels after liver transection could be confirmed.

Recently, doubts have arisen concerning the contribution of ischemia- reperfusion to total hepatocellular damage caused by liver surgery. Several experimental studies confirm our observation that mobilization of the liver results in profound liver tissue damage, even before inflow occlusion is applied[13, 30]. It is hypothesized that liver manipulation results in a disturbed hepatic microcirculation leading to hypoxia and Kupffer cell activation which causes additional hepatic injury[31]. Therefore, interventions aiming at minimization of hepatocellular damage should be started early during liver surgery.

Our study has some limitations. First, the relation between hepatocellular damage reflected by L-FABP or aminotransferases and postoperative outcome is indistinct. The current trial was powered to detect a 100 per cent difference in L-FABP level between the 15IPM and 30IPM group. In order to study differences in postoperative morbidity, a considerably larger number of patients should have been included[27, 32]. Furthermore, our results may not apply to patients with underlying liver disease. Insights in the effect of IPM on hepatocellular damage in these patients were reported recently[9]. After liver surgery with inflow occlusion, patients with liver cirrhosis had a lower difference between preoperative and maximum postoperative ASAT and ALAT levels compared with patients with normal liver, while their postoperative remnant liver function was worse. This further underlines the idea that aminotransferases, measured during hospital stay, may not accurately reflect the course of events secondary to hepatic inflow occlusion. Finally, a relation between prolonged pedicle clamping and accelerated outgrowth of residual liver disease was demonstrated in a murine model[33]. Clinical data on this topic in man are contradictory[34, 35]. Long-term results of a randomized controlled study that compared liver surgery with or without IPM showed no difference in disease-free or overall survival between the two patient groups[36].

To conclude, complete IPM using 30 min ischemic intervals resulted in similar hepatocellular damage, reflected by the highly sensitive damage marker L-FABP, compared with complete IPM using 15 min ischemic intervals, without induction of intestinal damage or loss of remnant liver function. If inflow occlusion during liver transection is necessary, intermittent pedicle clamping using 30 min ischemic intervals may be used.

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Part III

Assessing outcomes of liver surgery: hepatic damage as a result of oxaliplatin-based chemotherapy

Chapter 7

Nodular regenerative hyperplasia secondary to neoadjuvant chemotherapy for colorectal liver metastases

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van den Broek MA, Olde Damink SW, Driessen A, Dejong CH, Bemelmans MH. Nodular regenerative hyperplasia secondary to neoadjuvant chemotherapy for colorectal liver metastases. *Case Reports Med* 2009; 2009: 457975.

ABSTRACT

Liver resection is the only curative treatment for patients with colorectal liver metastases (CLMs). Chemotherapy can improve resectability rates, but has a potential harmful effect on the non-tumour-bearing liver. Patients with chemotherapy-induced hepatic injury undergoing major liver surgery have higher risks of post-resectional morbidity. We present two cases of patients without pre-existent liver disease treated with oxaliplatin-based chemotherapy followed by surgical resection of their CLMs. Their intra-operative liver specimen showed morphologic abnormalities characteristic of nodular regenerative hyperplasia (NRH). NRH led to portal hypertension in both patients that resulted in deleterious post-resectional complications and death of one patient. Interestingly, the other patient underwent two repeat non-anatomic liver resections because of recurrent CLMs. The intra-operative liver specimen still showed signs of NRH and sinusoidal congestion, but the post-resectional courses were uneventful. Nevertheless, caution is recommended in patients with suspected NRH. Careful volumetric analysis should guide the operative strategy. When future remnant liver volume or function are regarded insufficient, portal vein embolization or restrictive surgery should be considered.

INTRODUCTION

Colorectal liver metastases (CLMs) develop in 50 to 60 per cent of patients with colorectal carcinoma[1]. Resection remains the only curative treatment, but just a fifth of patients are initially eligible for resection. In these patients, perioperative chemotherapy has proven to elongate progression-free survival[2]. In addition, administration of chemotherapy can improve resectability in 15 to 30 per cent of patients with initially unresectable disease[1, 3]. However, recent reports show a potential harmful effect of oxaliplatin and irinotecan-based neoadjuvant chemotherapy on the non-tumour-bearing liver[4-6]. Patients with histologically proven chemotherapy-related hepatic injury undergoing major liver surgery have higher risks of post-resectional morbidity and mortality[6, 7].

Here, we present two cases of patients without pre-existent liver disease preoperatively treated with oxaliplatin and capecitabine because of CLMs, who developed nodular regenerative hyperplasia (NRH) of the liver causing deleterious complications after major hemihepatectomy. Interestingly, one of these patients underwent limited repeat resections after 12 and 18 months because of recurrent CLMs, after which the clinical courses were uneventful.

PATIENT CASES

Case 1

A 68-year-old man was referred to our hospital because of synchronous CLMs. His medical history revealed a colorectal carcinoma (pT3N0M1) treated by right hemicolectomy. Neoadjuvant chemotherapy consisting of 6 cycles of oxaliplatin (130 mg/m^2) and capecitabine (1000 mg/m^2 two-times daily) was initiated after resection of the colorectal primary. The patient had no history of liver disease and computed tomography (CT) scan did not reveal any signs of cirrhosis, portal hypertension, parenchymal abnormalities or other liver pathology. Liver function tests prior to and after administration of chemotherapy are depicted in Table 7.1. CT images showed a partial response of the CLMs with metastases remaining in segments IVb, VI and VIII for which an extended right hemihepatectomy was the only realistic curative treatment. As a remnant liver volume of only 18 per cent was calculated by CT volumetry, preoperative right portal vein embolization was performed. Five weeks later, remnant liver volume increased up to 28 per cent which was regarded just sufficient for safe hemihepatectomy. An extended right hemihepatectomy with Roux-Y reconstruction was performed as detailed previously[8]. A bluish appearance of the liver was noticed intra-operatively without signs of portal hypertension. Histological examination of the liver specimen showed sinusoidal congestion and nodularity characteristic of NRH. The postoperative course was uncomplicated

Table 7.1 Laboratory values of the patients described in case 1 and 2 before and after administration of oxaliplatin-based neoadjuvant chemotherapy.

	Case 1		Case 2	
	Before	After	Before	After
Alkaline phosphatase (IU/L)	205	237	328	205
γ-glutamyltransferase (IU/L)	50	42	233	90
ASAT (IU/L)	n.a.	59	61	79
ALAT (IU/L)	n.a.	23	91	57
Bilirubin (total) (μmol/L)	n.a.	39	12	21
INR	n.a.	n.a.	n.a.	n.a.

ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase; INR, international standardized ratio; n.a., not available.

till day four. From then on, bilirubin level and prothrombin time increased reflecting post-resectional liver failure. Acute upper gastro-intestinal hemorrhage occurred on day eight for which early management consisted of emergent endoscopy that showed bleeding from grade IV gastro-oesophageal varices. A CT scan performed on the same day showed impressive portacaval collateral shunting in the splanchnic area (Figure 7.1). Unfortunately, hemorrhage recurred several hours later and a second endoscopy failed to control it. Therefore, a Sengstaken-Blakemore tube was inserted and a laparotomy was performed during which the stomach was packed. During relaparotomy, no evidence of liver surgery-related complications was found. Because of ongoing deterioration, a distal splenorenal shunt was created one day later to relieve portal pressure.

Despite these efforts, the patient died several hours after relaparotomy because of multi-organ failure secondary to hemorrhagic shock. Post-mortem evaluation revealed NRH of the liver combined with hepatic congestion and infarction as well as extensive collateral vessels in the splanchnic region. Again, there was no evidence of surgery-related technical failure such as portal or hepatic vein obstruction.

Case 2

A 51-year-old man consulted our hospital because of synchronous CLMs after sigmoid resection for colorectal carcinoma (pT3N0M1) elsewhere. The patient received six cycles of oxaliplatin (130 mg/m^2) and capecitabine (1000 mg/m^2 two-times daily) subsequent to resection of his primary tumour. There was no history of liver disease. The evolution of the liver function tests of the patient described in this case is shown in Table 7.1. CT imaging showed tumour regression after chemotherapy with metastases remaining in segments III, IVa and the right hemi-liver. A right hemihepatectomy with metastasectomy of tumour from segments III and IVa was performed without biliary reconstruction[8]. Remnant liver volume was estimated intra-operatively to be 35 per cent with a bluish appearance of the liver remnant. Histological examination of the liver specimen showed

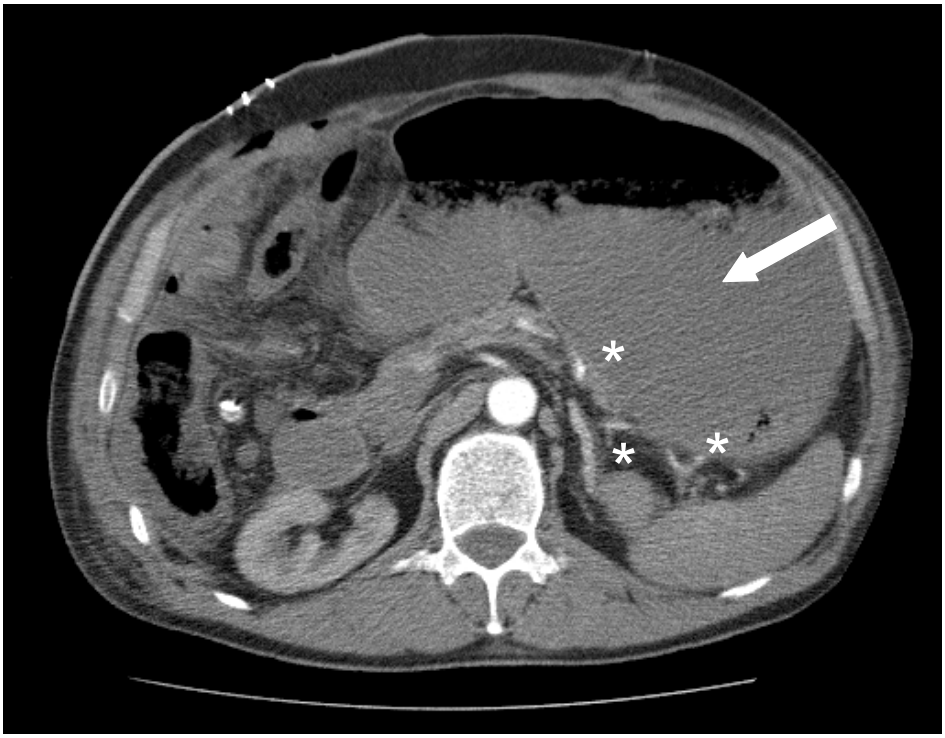

Figure 7.1 Contrast-enhanced CT scan performed 8 days postoperatively (case 1). The stomach (white arrow) is filled with blood after upper gastro-intestinal bleeding from gastro-oesophageal varices secondary to portal hypertension. Multiple, large collateral veins run along the stomach and spleen (white asterisk).

a nodular appearance with hepatic congestion characteristic of NRH (Figure 7.2 and 7.3). The postoperative course was complicated by bile leakage treated by CT guided percutaneous drainage and ERCP guided common bile duct stenting. Bilirubin level and prothrombin time decreased during admission and the patient was discharged 14 days postoperatively. Two days after discharge, the patient was readmitted in his hometown hospital because of portal hypertension leading to oesophageal variceal bleeding with hepatic encephalopathy treated by endoscopic band ligation and conservative measures, respectively. Laboratory values normalized gradually consistent with an improvement of the patient's clinical and mental status. Full recovery was accomplished after two months.

One year afterwards, the patient presented himself with multiple, recurrent CLMs in segments II and III for which he received 3 cycles of irinotecan (300 mg/m^2) prior to liver surgery. Preoperative diagnostic liver biopsy showed minimal nodularity and steatosis without indications for NRH or steatohepatitis. The patient underwent treatment of the CLMs by non-anatomical wedge resections followed by an uneventful clinical course. Histopathological examination of intra-operative liver specimen showed signs consistent with NRH again.

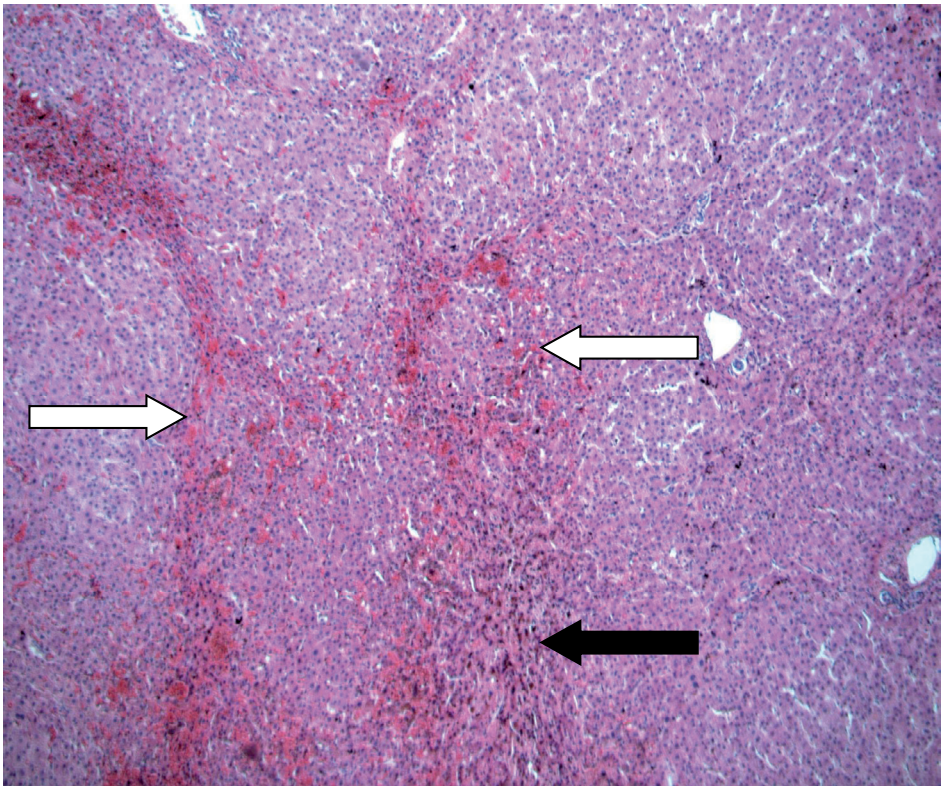

Figure 7.2 Overview of the intra-operatively obtained liver specimen (case 2) in which the liver has a disturbed architecture with nodular appearance of liver parenchyma (white arrows) characteristic of nodular regenerative hyperplasia. Areas with sinusoidal congestion are also present (black arrow). Haematoxylin and eosin, original magnification x50.

Eighteen months later, another recurrent CLM was resected from segment IVb after which the clinical course was uneventful. No neoadjuvant chemotherapy was administered prior to surgery this time as the CLM was deemed resectable without the need for downsizing by means of chemotherapy. Histopathological examination of the non-tumour-bearing liver did not reveal signs for NRH anymore. However, some areas of the non-tumour-bearing liver did still show minimal sinusoidal congestion.

DISCUSSION

Chemotherapy is an essential element in the multimodal approach to CLMs. However, chemotherapy consisting of either irinotecan or oxaliplatin has been associated with the development of histological lesions in the non-tumour-bearing liver that are related to post-resectional complications[2, 6, 7]. Irinotecan has been shown to induce hepatic inflam-

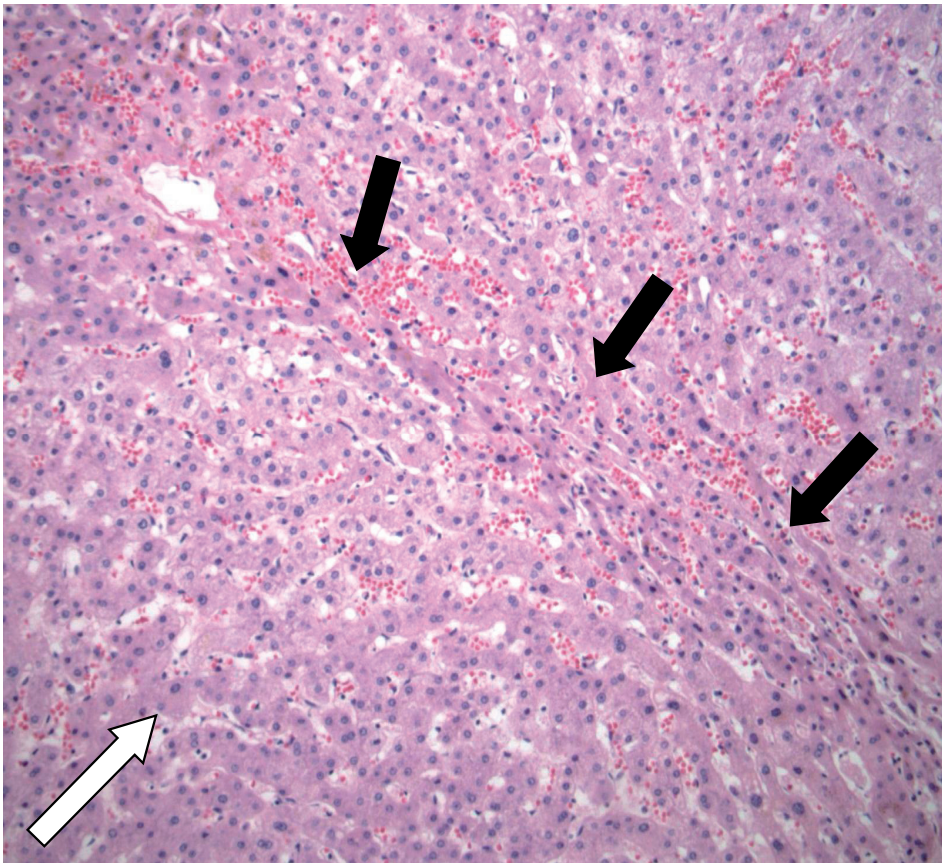

Figure 7.3 Detail of the intra-operative liver specimen (case 2) showing nodular regenerative hyperplasia, in which a regenerative nodule (white arrow) is bordered by irregular aligned, small-sized hepatic trabeculae (black arrows). Haematoxylin and eosin, original magnification x100.

mation classified as chemotherapy-associated steatohepatitis which is associated with an increased 90-day mortality after liver surgery[6, 9]. Oxaliplatin is related to vascular lesions classified as sinusoidal obstruction syndrome (SOS) and, sporadically, also to NRH[4, 5, 7, 10, 11]. Recently, Nakano and colleagues showed a significant association between the presence of vascular lesions in the non-tumour-bearing liver secondary to oxaliplatin and increased morbidity after major hemihepatectomy[7].

NRH of the liver is characterized by the diffuse presence of regenerative nodules made up of hyperplastic hepatocytes less than 3 mm in diameter without fibrous septa[12]. The liver parenchyma between the nodules contains atrophic hepatocytes and shows signs of sinusoidal dilatation and congestion (Figure 7.2 and 7.3). Usually, these lesions are clinically asymptomatic but they can be associated with portal hypertension, splenomegaly and bleeding from oesophageal varices[13]. Liver function is usually preserved

in patients suffering from NRH, although slight increases in alkaline phosphatase or aspatate aminotransferase might be noticed.

The parenchymal injury characteristic of NRH originates from a heterogeneous perfusion of the liver secondary to obliterative lesions in either the central vein or hepatic sinusoids[12, 14]. Oxaliplatin-based chemotherapy has a toxic effect on sinusoidal endothelial cells resulting in sinusoidal dilatation, congestion and obstruction. Depletion of sinusoidal glutathione and activation of matrix metalloproteinases by oxaliplatin have been postulated as pathogenic factors in the development of these sinusoidal lesions[14]. Furthermore, it has been hypothesized that the sinusoidal lesions aggravate portal pressure and impair hepatic regeneration[5, 15]. The extent of reversibility of these lesions is still uncertain; patients undergoing repeat resection because of recurrent CLMs still showed sinusoidal dilatation or (progressive) fibrosis[5]. On the other hand, a longer interval between neoadjuvant chemotherapy and surgical resection seemed to decrease the incidence of sinusoidal injury[7].

It is imperative to recognize the presence of SOS or NRH prior to hemihepatectomy. The diagnostic value of a preoperative liver biopsy seems to be minimal because of sampling error resulting in a high false-negative result rate and therefore, suspicion is merely based on the patient's history, preoperative liver function and intra-operative macroscopic aspect. In this respect, several factors independently associated with sinusoidal injury have been identified[7]. These include female gender, administration of 6 or more cycles of oxaliplatin-based chemotherapy, abnormal value of preoperative aspartate aminotransferase (above 36 IU/L) and indocyanine green retention rate at 15 minutes of more than 10 per cent. Vauthey and colleagues suggested to perform a preoperative, diagnostic laparoscopy to identify a bluish appearance of the liver[6].

It could be postulated that, considering the hepatotoxicity of neoadjuvant chemotherapy, initial liver surgery followed by adjuvant chemotherapy might be the treatment of choice in patients with clearly resectable CLMs[16]. However, the effects on disease-free and long-term survival should be evaluated in adequately powered, randomized controlled trials. In case of unresectable or recurrent CLMs preoperatively treated with oxaliplatin-based chemotherapy, caution is recommended when an extensive hepatic resection is scheduled and SOS or NRH are suspected. Even if the interval between chemotherapeutic treatment and surgical resection is long, SOS or NRH might still be present. Careful analysis of future remnant liver volume and function should guide the operative strategy. Either preoperative portal vein embolization and/or restrictive surgery should be considered when future remnant liver volume or function are regarded insufficient. The safety limit of future remnant liver volume after neoadjuvant chemotherapy treatment would be between 30 and 40 per cent; however, this number needs careful prospective validation.

Future research should focus on assessment of the optimal duration of chemotherapeutic treatment and timing of liver surgery as well as the development of reliable screening methods for chemotherapy-associated hepatotoxicity and strategies to protect the non-tumour-bearing liver from the deleterious effects of neoadjuvant chemotherapy.

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Chapter 8

Hyaluronic acid as a marker of hepatic sinusoidal obstruction syndrome secondary to oxaliplatin-based chemotherapy in patients with colorectal liver metastases

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ABSTRACT

Background

A considerable number of patients develop sinusoidal obstruction syndrome (SOS) after oxaliplatin-based chemotherapy for colorectal liver metastases (CLMs). SOS is associated with adverse outcomes after major hepatectomy. Hyaluronic acid (HA) is a marker of hepatic sinusoidal endothelial cell function and may serve as an accurate marker of SOS. This study aimed to assess the value of systemic HA levels and fractional extraction (FE) of HA by the splanchnic area and liver as markers of SOS after oxaliplatin-based chemotherapy for CLMs.

Methods

Forty patients were studied. The presence of SOS was assessed histopathologically. Blood samples from the radial artery, portal and hepatic vein were collected. HA levels were determined by ELISA and the FE of HA was estimated.

Results

SOS was present in 23 patients, of whom 11 showed moderate or severe SOS. Preoperative HA levels were significantly higher in patients with moderate or severe SOS (Group B, $n = 11$) compared to patients with no or mild SOS (Group A, $n = 29$) (51.6±10.2 ng/mL versus 32.1±3.5 ng/mL, p=0.030). A cutoff HA level of 44.1 ng/mL yielded a sensitivity of 67 per cent and specificity of 83 per cent for detection of SOS. The positive predictive value was 50 per cent and negative predictive value 91 per cent. Both groups showed a similar FE of HA by the splanchnic area (−7.9±8.5 per cent in Group A versus 7.3±3.6 per cent in Group B, p=0.422) and liver (−10.7±6.2 per cent in Group A versus 4.6±2.3 per cent in Group B, p=0.265).

Conclusion

Systemic HA levels can be used to detect patients at risk of SOS after oxaliplatin-based chemotherapy for CLMs. Additional investigations into the presence of SOS are indicated in patients with elevated HA levels.

INTRODUCTION

A multidisciplinary approach consisting of chemotherapy followed by surgery is being used increasingly to treat patients with colorectal liver metastases (CLMs). This strategy has proven to enhance resectability rates and increase progression-free survival in resected patients[1-3]. However, treatment with oxaliplatin-based chemotherapy is held responsible for the development of injury to hepatic sinusoidal endothelial cells (SECs), which may manifest as sinusoidal obstruction syndrome (SOS)[4, 5]. The presence of SOS has been reported in approximately half of patients treated with oxaliplatin and adversely affects post-resectional outcomes, especially after major liver resection[1, 5-11]. In patients scheduled to undergo major liver surgery, information on the presence of SOS *prior* to surgery is important as it may alter the surgical strategy.

The gold standard for the diagnosis of SOS is measurement of the wedged hepatic venous pressure gradient and assessment of liver pathology[12]. The limitations of obtaining a preoperative liver biopsy are well established, including the risk of needle-track deposits, sampling error, and morbidity associated with the invasive procedure[13, 14]. Furthermore, inter- and intraobserver variability in the histopathological assessment of SOS is a problem. Hence, there is a need for a non-invasive, reliable and reproducible screening tool for SOS.

To date, non-invasive detection methods of SOS include elevated ASAT to platelet ratio index (APRI-score), increased spleen size determined by CT volumetry, or increased indocyanine green retention rate. These are all indirect markers of sinusoidal injury and have modest sensitivity and specificity for the diagnosis of SOS[6, 10, 15, 16]. As damage to hepatic SECs is a primary event in the development of SOS, markers for endothelial cell injury may have a better diagnostic accuracy[17]. One of these markers is hyaluronic acid (HA)[18-20]. HA is synthesized by cells of mesodermal lineage. Part of HA turnover occurs by local breakdown. The remainder is transported to the circulation, where approximately 90 per cent is cleared by a receptor-facilitated mechanism almost solely present in hepatic SECs[18, 21, 22]. Systemic HA levels are thus regarded as a direct marker of hepatic SEC function and have been used for the non-invasive detection of hepatic SEC damage in a variety of clinical conditions[18, 20, 23].

When SOS is diagnosed *prior* to liver surgery, timely adjustments to the operative plan can be made. Strategies such as portal vein occlusion and/or two-stage hepatectomy can be applied to enhance or preserve future remnant liver volume and function[15, 24]. This study aimed to assess the value of systemic HA levels and fractional extraction (FE) of HA by the splanchnic area and liver as markers of SOS after oxaliplatin-based chemotherapy in patients with CLMs.

METHODS

Patient selection

Consecutive patients undergoing partial hepatectomy for CLMs at Maastricht University Medical Centre between January 2008 and December 2009 were prospectively included. Exclusion criteria were (a) laparoscopic liver resection, (b) no oxaliplatin-based chemotherapy or interval between chemotherapy and liver surgery more than 12 months, (c) repeat liver surgery, (d) liver tissue or plasma not suitable for analysis, and (e) presence of inflammatory joint disease. The study was performed in accordance with the ethical standards of the Declaration of Helsinki.

Protocols of chemotherapy

Patients presenting with CLMs were discussed in our multidisciplinary oncology team. On indication, patients received bevacizumab as 7.5 mg/kg and oxaliplatin as 130 mg/m^2 intravenous infusion on day 1 combined with oral capecitabine two-times daily dosed 1000 mg/m^2 on day 1-14, followed by a treatment-free week (day 15-21). After 3 cycles, eligibility for and optimal timing of surgery was determined based on the radiological tumour response[25]. The waiting time between end of chemotherapy and surgery was kept at a minimum of 4 weeks.

Preoperative management

Standard laboratory tests were obtained preoperatively from the clinical chemistry laboratory. The APRI-score was calculated using the following formula: APRI-score = [(ASAT / upper limit of normal of ASAT) / platelet count]*100 per cent[26]. The upper limit of normal of ASAT was 30 IU/L. Data on patient demographics, extent of liver disease, chemotherapy regimens, and interval between chemotherapy and surgery were collected.

Surgical procedure

Surgical procedures were performed as described before[27]. In short, patients routinely had indwelling catheters in a jugular vein and radial artery. After liver mobilization, an ultrasound was performed which guided the operative strategy. A Cavitron Ultrasonic Surgical Aspirator (CUSA system 200 macrodissector, Cavitron Surgical Systems, Stamford, CT, USA) and Argon beam coagulation (Force GSU System, Valleylab, Boulder, CO, USA) were used for liver transection.

Postoperative care

The postoperative care was according to an enhanced recovery after liver surgery programme[27]. Liver surgery-related complications were recorded prospectively. These

included bile leakage, ascites, post-resectional liver failure, intra-abdominal hemorrhage, intra-abdominal abscess and operative mortality with a Clavien-Dindo grade of 3 or more and occurring within 90 days after surgery[28, 29].

Blood sampling

Blood was sampled from the radial artery before the start of surgery. In a subgroup, additional samples were collected intra-operatively from the radial artery, portal vein and hepatic vein as described in [30]. Blood samples were transferred to prechilled heparin-coated blood collection tubes and put on ice. Plasma was prepared by centrifugation at 4°C at 3500 x g for 15 min after which supernatant was stored in aliquots at –80°C till analysis.

Tissue sampling and histopathological analysis

After resection, the specimen was sliced and fixed in 4 per cent buffered formaldehyde. Tissue samples were taken from the non-tumour-bearing liver at a minimum distance of 2 cm from the tumour while avoiding the subcapsular region. They were routinely embedded in paraffin and 4 µm sections were cut. Sections were examined by two experienced HPB pathologists unaware of the clinical data. In case of discrepancy, the section was discussed and consensus was reached. Morphological analysis was based on haematoxylin and eosin, reticulin and Trichrome Masson stains. Histopathological features containing vascular and parenchymal changes were scored according to the classification of Rubbia-Brandt and colleagues[5]. Sinusoidal dilatation was graded as follows: grade 0. no dilatation, grade 1. centrilobular dilatation in zone 3 [mild SOS], grade 2. centrilobular dilatation in zones 2 and 3 [moderate SOS], and grade 3. panlobular sinusoidal dilatation and bridging congestion [severe SOS]. For analysis, two groups were created: group A consisted of no lesions and grade 1 lesions and group B of grade 2 and 3 lesions, as described earlier in [6, 11]. Peliosis and nodular regenerative hyperplasia (NRH) were scored as present or absent.

Systemic hyaluronic acid level as a marker of SOS

HA levels were determined using a commercially available ELISA kit (Corgenix Inc., Broomfield, CO, USA), which had a lower limit of detection of 10 ng/mL. According to the manufacturer, HA levels in a healthy population ranged between 0-75 ng/mL. Samples below the detection limit were excluded.

Fractional extraction of hyaluronic acid

FE of HA by the splanchnic area ($FE_{splanchnic}$) and liver (FE_{liver}) was calculated using the following formulas: $FE_{splanchnic}$= [(hepatic vein level – radial artery level) / radial artery level]*100 per cent and FE_{liver} = [(hepatic vein level – (0.3*radial artery level + 0.7*portal

vein level)) / (0.3*radial artery level + 0.7*portal vein level)]*100 per cent. The FE_{liver} represents the percentage of HA that is actually taken up by the liver in relation to its inflow. Since we were unable to measure portal vein and hepatic artery flow, we assumed that approximately 30 per cent of hepatic inflow was derived from the hepatic artery and 70 per cent from the portal vein (see [31]). Positive values represent net release and negative values net uptake.

Statistical analysis

Data are given as mean with standard error or median with range. For continuous data, statistical analyses were performed using a student *t* test or Mann-Whitney U test, depending on the nature of the data. Differences between dichotomous variables were calculated with Pearson's chi-square test. The FE of HA was tested using a one-sample t-test with a theoretical mean of zero. A receiver operating characteristic curve was created to determine the clinical performance and optimal diagnostic cutoff value of systemic HA levels. Analyses were performed using SPSS version 15.0 for Windows (SPSS Inc. Chicago, IL, USA). A p-value below 0.050 was considered significant.

RESULTS

Patient characteristics

During the study period, 108 patients underwent a partial hepatectomy, of whom 40 were eligible for inclusion (Figure 8.1). Their characteristics are described in Table 8.1. The median number of oxaliplatin cycles was 5 (2-10) with a median interval between oxaliplatin and surgery of 61 days (26-338 days). In 35 patients (88 per cent), bevacizumab was added to oxaliplatin.

Surgical procedure

The operative strategy was a liver-first approach in 9, a conventional approach in 30, and a two-stage approach in 1 patient, respectively[32]. A median of 3 liver segments (range 1-5) was resected. Additional operative characteristics are depicted in Table 8.2.

Incidence of hepatic SOS

SOS was present in 23 patients (58 per cent). Mild SOS was seen in 12 (30 per cent), moderate in 9 (23 per cent) and severe in 2 patients (5 per cent), respectively. Furthermore, 2 patients (5 per cent) showed peliosis, both with the presence of moderate or severe SOS. Patients with different SOS grade were divided into group A, consisting of 29 patients with no or mild sinusoidal dilatation, and group B, consisting of 11 patients with moderate or severe sinusoidal dilatation. There was no difference between group A and

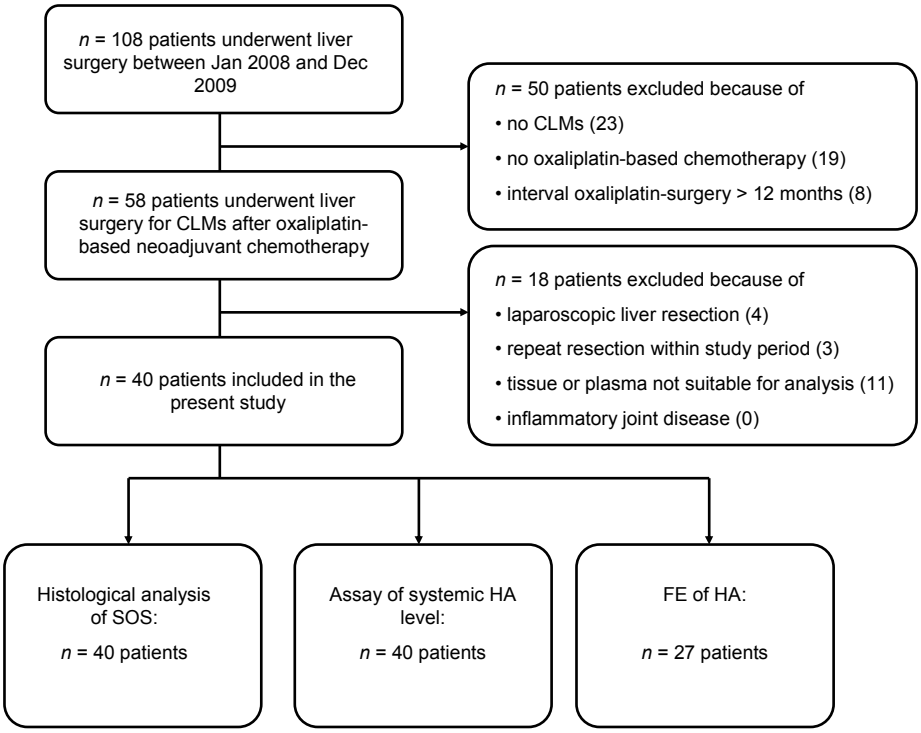

Figure 8.1 Flowchart of patients.
Legend: CLM, colorectal liver metastasis; HA, hyaluronic acid; SOS, sinusoidal obstruction syndrome; FE, fractional extraction.

Table 8.1 Patient characteristics.

	***N* = 40**
Baseline characteristics	
Age (years)	61 ± 2
Sex (male)	20 (50)
BMI (kg/m^2)	26 ± 1
ASA classification	
ASA I	5 (13)
ASA II	31 (78)
ASA III	4 (10)
Primary disease	
Colon	20 (50)
Rectum	20 (50)
Colorectal liver metastases	
Synchronous	31 (78)
Metachronous	6 (15)

Table 8.1 Continued

Recurrent	3 (8)
Median no. of metastases (range)	2 (1-10)
Size of largest hepatic metastases (mm)	23 ± 3
Presence of extra-hepatic disease	5 (13)
Preoperative liver function	
ASAT (IU/L)	27 ± 2
ALAT (IU/L)	32 ± 2
Total bilirubin (μmol/L)	14 ± 1
INR	1 ± 0
APRI-score	0.46 ± 0.05
Chemotherapy regimens	
CAPOX	5 (13)
CAPOX + bevacizumab	32 (80)
CAPOX + CAPIRI + bevacizumab	3 (8)
Amount of chemotherapy	
Oxaliplatin	
• Median no. of cycles (range)	5 (2-10)
• Median cumulative dose (mg/m^2) (range)	650 (240-1300)
• Median interval chemotherapy – surgery (days)	61 (26-338)
Bevacizumab	
• Median no. of cycles (range)	3 (1-7)
• Median cumulative dose (mg/kg) (range)	22.5 (7.5-52.5)
• Median interval chemotherapy – surgery (days)	79 (26-359)
Irinotecan	
• Median no. of cycles (range)	3 (1-6)
• Median cumulative dose (mg/m^2) (range)	1050 (350-2800)
• Median interval chemotherapy – surgery (days)	129 (67-364)

Numbers indicate mean ± standard error or absolute number (percentage) unless otherwise indicated; *N* and no., number; ASA, American Society of Anaesthesiologists; BMI, body mass index; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase; INR, international standardized ratio; APRI-score, ASAT to platelet ratio index; CAPOX, capecitabine + oxaliplatin; CAPIRI, capecitabine + irinotecan.

group B with respect to number of oxaliplatin cycles, interval between oxaliplatin and surgery or addition of bevacizumab (Table 8.3). Also APRI-scores, which were available in 16 patients, were comparable between the groups. In addition, there was no relation between the presence of SOS and adverse post-resectional outcome.

Systemic hyaluronic acid level as a marker of SOS

Mean systemic HA levels before the start of surgery were significantly higher in patients with moderate or severe SOS compared to patients with no or mild SOS (51.6±10.2 ng/

Table 8.2 Operative characteristics and pathological findings.

	***N* = 40**
Surgery	
Type of resection[#]	
Hemihepatectomy	15 (38)
Central bisectionectomy	5 (13)
Bisegmentectomy	4 (10)
Unisegmentectomy	4 (10)
Non-anatomical resection	12 (30)
Median no. of resected segments (range)	3 (1-5)
Adjuvant techniques	
Portal vein ligation	3 (8)
Two-stage hepatectomy	1 (3)
Median blood loss (mL) (range)	750 (200-11000)
Red blood cell transfusion	12 (30)
Duration of surgery (min)	252 ± 14
Pathology*	
Sinusoidal dilatation	
No	17 (43)
Mild (grade 1)	12 (30)
Moderate (grade 2)	9 (23)
Severe (grade 3)	2 (5)
Peliosis	2 (5)
Nodular regenerative hyperplasia	11 (28)

Numbers indicate mean ± standard error or absolute number (percentage) unless otherwise indicated; [#] According to the IHPBA nomenclature[44]; * graded according to Rubbia-Brandt and colleagues[5]; *N*, number.

Table 8.3 Comparative analysis of baseline, operative and postoperative characteristics between patients with no or mild SOS (Group A) and moderate or severe SOS (Group B).

	Group A	**Group B**	**p-value**
	n = 29	*n* = 11	
Baseline characteristics			
Age (years)	62 ± 2	59 ± 3	0.463
Sex (male)	13 (45)	7 (64)	0.288
BMI (kg/m^2)	25 ± 1	27 ± 1	0.270
Median no. of CLMs	2 (1-10)	2 (1-5)	0.751
Size of largest CLM (mm)	23 ± 4	22 ± 5	0.974
Presence of extra-hepatic disease	5 (17)	0 (0)	0.538
Preoperative liver function			
ASAT (IU/L)	29 ± 2	23 ± 4	0.177
ALAT (IU/L)	34 ± 3	25 ± 5	0.092

Table 8.3 Continued

	Group A	Group B	p-value
Bilirubin (µmol/L)	13 ± 1	15 ± 1	0.257
INR	1 ± 0	1 ± 0	0.860
Markers of SOS			
Hyaluronic acid (ng/mL)	32.1 ± 3.5	51.6 ± 10.2	0.030
APRI-score*	0.42 ± 0.07	0.61 ± 0.18	0.254
Fractional extraction of hyaluronic acid			
Splanchnic area (%)	−7.9 ± 8.5	7.3 ± 3.6	0.422
Liver (%)	−10.7 ± 6.2	4.6 ± 2.3	0.265
Chemotherapy regimens			
Bevacizumab-containing regimen	26 (90)	9 (82)	0.503
Median no. cycles oxaliplatin	5 (3-9)	5 (2-10)	0.580
Median interval oxaliplatin (days)	61 (26-338)	61 (39-253)	0.940
Surgery			
Median no. resected segments	3 (1-5)	3 (1-4)	0.704
Median blood loss (mL)	700 (200-11000)	900 (200-2200)	0.693
Duration of surgery (min)	249 ± 16	258 ± 27	0.775
Postoperative outcome			
Liver surgery-related complications	8 (28)	3 (27)	0.984
Operative mortality	2 (7)	0 (0)	
Bile leakage	5 (17)	2 (18)	
Ascites	2 (7)	0 (0)	
Post-resectional liver failure	1 (3)	0 (0)	
Intra-abdominal abscess	6 (21)	2 (18)	
Intra-abdominal hemorrhage	0 (0)	0 (0)	
Median length of stay (days)	8 (5-33)	8 (5-38)	0.329

Numbers indicate mean ± standard error or absolute number (percentage) unless otherwise indicated; *N*, number; BMI, body mass index; CLM, colorectal liver metastasis; SOS, sinusoidal obstruction syndrome; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase; INR, international standardized ratio; APRI-score, ASAT to platelet ratio index; * only available in 16 patients.

mL versus 32.1±3.5 ng/mL, p=0.030). The area under the curve of systemic HA levels for identification of SOS was 0.736 (95 per cent confidence interval 0.490-0.992, p=0.078). An optimal cutoff value of 44.1 ng/mL yielded a sensitivity of 67 per cent and specificity of 83 per cent for detection of moderate or severe SOS. The positive predictive value of this level was 50 per cent and negative predictive value 91 per cent.

Fractional extraction of hyaluronic acid

In 27 patients, intra-operative samples for the calculation of the FE of HA were available. Mean HA levels in the radial artery, portal and hepatic vein did not differ significantly

between Group A and B at this time point (level of significance not shown) (Figure 8.2). The $FE_{splanchnic}$ of HA was comparable between Group A and B (−7.9±8.5 per cent versus 7.3±3.6 per cent, p=0.422). The FE_{liver} of HA was −10.7±6.2 per cent in patients Group A, representing a trend towards net hepatic uptake (p=0.087 from zero). In patients in Group B, FE_{liver} of HA was 4.6±2.3 per cent, representing a non-significant net hepatic release (p=0.176 from zero). The difference in FE_{liver} did also not reach statistical significance (p=0.265).

DISCUSSION

In the present study, fifty-eight per cent of patients showed histological evidence of SOS. Systemic HA levels were significantly elevated in patients with moderate or severe SOS compared to patients with no or mild SOS. The diagnostic accuracy of systemic HA levels for the detection of SOS was fair. Although there seemed to be a trend towards net hepatic uptake in patients with no or mild SOS versus net hepatic release in patients with moderate or severe SOS, the FE of HA was not significantly different between these groups.

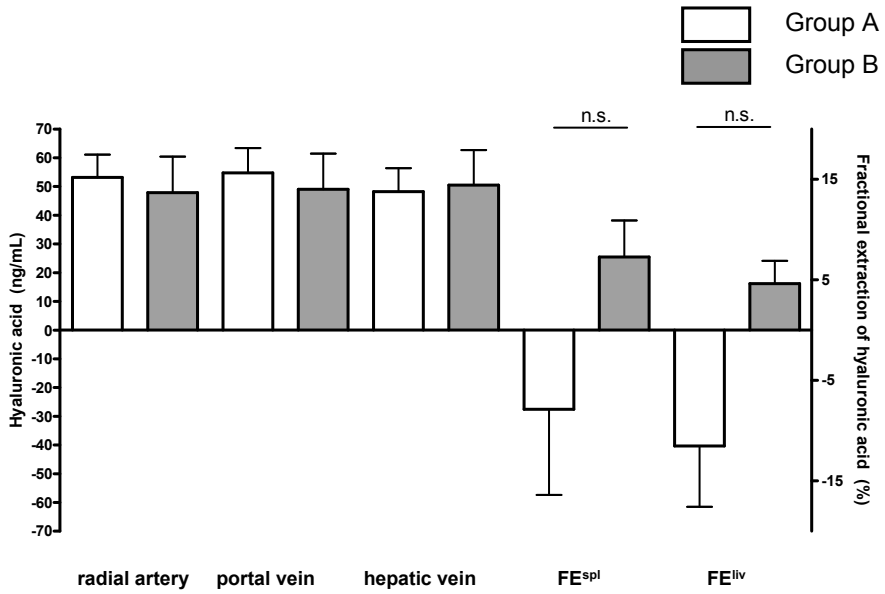

Figure 8.2 Intra-operative HA levels in the radial artery, portal vein and hepatic vein and fractional extraction of HA by the splanchnic area and liver in patients with no or mild SOS (Group A) and with moderate or severe SOS (Group B).
Legend: HA, hyaluronic acid; FE_{spl}, fractional extraction by the splanchnic area; FE_{liv}, fractional extraction by the liver; SOS, sinusoidal obstruction syndrome; n.s., not significant.

The prevalence of SOS in our cohort was in the same range as data reported by other groups[5-8, 10, 33]. Surprisingly, we found no association between the presence of SOS and the number of oxaliplatin cycles, interval between oxaliplatin and surgery or addition of bevacizumab[5, 6, 10]. This may either be explained by an inter-individual variation in susceptibility to developing SOS or the small number of patients included in the present study.

To the best of our knowledge, this is the first study analyzing the value of systemic HA levels for the detection of SOS in patients with CLMs. Observed HA levels, although significantly elevated in patients with SOS, were within the normal range as was the optimal cutoff level for identification of patients with advanced SOS. These results may indicate that SEC injury induced by oxaliplatin was less severe than injury induced by other toxic substances, such as myeloablative conditioning therapy. In the latter condition, patients show HA levels which are on average tenfold higher than the upper limit of normal[20].

Increased systemic HA levels can either be due to decreased extraction or to increased production. Although there seemed to be a trend towards net uptake in patients with no or mild SOS versus net release in patients with moderate or severe SOS by the splanchnic area and liver, our attempt to estimate the FE of HA was not successful in detecting a significant difference. The reason for this remains speculative, but this observation may be due to the large variation in the FE, making our study underpowered to detect a significant difference. Otherwise, our assumption of the 30 per cent versus 70 per cent distribution of inflow may be incorrect, though FE by the splanchnic area, which does not rely on this assumption, showed the same trend.

The comparable FE of HA may as well reflect the actual fact that the net functional capacity of hepatic SECs for extraction of HA was not disturbed at the time of liver surgery, despite histological evidence of SOS. This could be related to (1) a recovery of SEC function during the interval between chemotherapy and surgery or (2) an altered intrahepatic blood flow secondary to sinusoidal injury, which is typically observed in patients suffering from NRH[5, 34, 35]. In patients with NRH, regions with hyperplastic hepatocytes alternate regions with hypoplastic hepatocytes as a result of a heterogenous perfusion secondary to SEC injury. Furthermore, our data point out that an increased release of HA by damaged SECs or enhanced production by hepatic stellate cells, which become activated in advanced SOS, is unlikely[36-38]. Taken together, the increased systemic HA levels in patients with SOS do not result from decreased hepatic uptake or increased hepatic release and may be explained by either portosystemic shunting due to portal hypertension or enhanced production at other sites than the liver. From a mechanistic point of view, it would be relevant to obtain more detailed insight into HA metabolism and SEC function in patients with SOS after oxaliplatin-based chemotherapy by either performing HA loading tests or PET scans with [^{11}C]hyaluronic acid[39, 40].

Other methods to predict SOS use indirect markers of SEC injury and may therefore reflect other disease states as well[6, 10, 11]. The APRI-scores did not differ significantly in the present study, but, as preoperative platelet counts were not available in the majority of patients, the diagnostic accuracy of HA could not be compared to that of the APRI-score. It is likely that a combination of biomarkers comprising genetic, functional, radiological and damage parameters would be required to accurately stratify patients[6, 16]. Circulating von Willebrand Factor seems a reasonable candidate marker based on recent pathophysiological insights derived from gene array studies in humans[41-43].

Our study has some limitations. The accuracy of the HA assay was moderate due to its relatively high lower limit of detection. Optimization of the assay protocol and validation of our results in a larger patient cohort are warranted. In addition, systemic HA levels were measured at the start of surgery only. Consequently, information is lacking on the direct toxic effect of oxaliplatin on hepatic SECs, taken into account the 60 day interval between chemotherapy and surgery. Serial sampling just before and after chemotherapy might shed a light on temporary SEC dysfunction after oxaliplatin. Finally, the assay of systemic HA levels yielded a high specificity and negative predictive value at the cutoff level of 44.1 ng/mL for the diagnosis of moderate or severe SOS. This implies that patients with low HA levels most probably do not suffer from sinusoidal injury. In patients with high HA levels, additional investigations are warranted.

To conclude, elevated systemic HA levels had a fair diagnostic performance for the detection of patients with moderate or severe SOS after oxaliplatin-based chemotherapy for CLMs. In patients with high HA levels scheduled to undergo major hepatectomy, preoperative assessment of liver pathology by a transjular liver biopsy and wedged hepatic venous pressure gradient measurement may be indicated in order to identify those who would benefit most from two-stage hepatectomy with portal vein occlusion.

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Chapter 9

Hepatic sinusoidal obstruction syndrome reduces the effect of oxaliplatin in patients with colorectal liver metastases

Adapted from:

Vreuls CP, van den Broek MA, Winstanley A, Koek GH, Wisse E, Dejong CH, Olde Damink SW, Bosman FT, Driessen A. Hepatic sinusoidal obstruction syndrome (SOS) reduces the effect of oxaliplatin in colorectal liver metastases. *Histopathology* 2012; 61: 314-318.

ABSTRACT

Background

Oxaliplatin is an important chemotherapeutic agent that is used to treat patients with colorectal liver metastases (CLMs). Its administration results in reduction of tumour load which makes curative resection possible in case of initially unresectable disease. Treatment with oxaliplatin-based chemotherapy has significant side effects, as oxaliplatin can induce sinusoidal obstruction syndrome (SOS) in the non-tumour-bearing liver. SOS is associated with increased post-resectional morbidity and decreased long-term survival. We hypothesized that SOS might impede hepatic perfusion, thereby interfering with the tumour environment and attenuating the tumour response to chemotherapy.

Methods

From the prospective liver database of Maastricht University Medical Centre, 50 consecutive patients with CLMs were selected. All patients received oxaliplatin-based neoadjuvant chemotherapy followed by partial hepatectomy. The tumour regression grade (TRG) of metastases and presence of SOS in the non-tumour-bearing liver were studied histopathologically according to standard scoring systems.

Results

Thirty-two patients (64 per cent) showed histopathological signs of SOS, classified as mild in 26 per cent and moderate-severe in 38 per cent. The response to treatment, as expressed by TRG, was grade 1 [no residual cancer] in 5 (10 per cent), grade 2 [rare residual cancer cells] in 7 (14 per cent), grade 3 [fibrosis outgrowing residual cancer cells] in 14 (28 per cent), grade 4 [residual cancer cells outgrowing fibrosis] in 16 (32 per cent) and grade 5 [absence of regressive changes] in 8 patients (16 per cent), respectively. Advanced SOS grade was associated with a higher TRG (p=0.016).

Conclusion

The presence of SOS goes along with a lower tumour response to oxaliplatin-based neoadjuvant chemotherapy. Sinusoidal injury may induce hepatic hypoperfusion and hypoxia, which possibly reduce the response to chemotherapy.

INTRODUCTION

In 2008, colorectal cancer was the second most commonly diagnosed cancer in females and the third in males, responsible for 1.2 million new cancer cases worldwide[1]. The prognosis of patients with colorectal cancer is mainly determined by the presence of metastatic disease. The liver is the most common site of metastases and at the time of diagnosis of the colorectal primary, 15 to 20 per cent of patients have already developed colorectal liver metastases (CLMs)[2, 3]. If all CLMs can be removed by surgery, a five-year overall survival rate of around 50 per cent can be achieved[4-6]. Unfortunately, most patients are initially not eligible for liver surgery with curative intent due to multiple factors, such as high number of metastases, large lesion size or bilateral involvement. Currently, patients with initially unresectable disease are treated with chemotherapy, aiming at downsizing CLMs to enable resection and achieve potential cure[7]. Adam and colleagues reported a success rate of this strategy in 12.5 per cent of patients with a 5-year survival rate of 33 per cent[8]. These results have been achieved by administration of combined chemotherapeutic regimen, consisting of fluorouracil, oxaliplatin and irinotecan[9]. These regimens reduce hepatic tumour load as evidenced by histopathological assessment of the tumour regression grade (TRG)[10-12].

Treatment with oxaliplatin-based chemotherapy is, however, accompanied by significant side effects. Oxaliplatin induces sinusoidal obstruction syndrome (SOS) in the non-tumour-bearing liver in the majority of patients[13-16]. SOS was formerly known as hepatic veno-occlusive disease (VOD), a condition that has been described in patients after myeloablative hematopoietic stem cell transplantation. Clinical features of patients with advanced VOD include hyperbilirubinemia (bilirubin level above 2 mg/dL), ascites, hepatomegaly, portal hypertension or weight gain[17]. These symptoms have also been described in patients with CLMs who received prolonged oxaliplatin-based chemotherapy treatment, but in the majority, SOS presents as a subclinical condition[18]. The presence of SOS is associated with higher post-resectional morbidity rates, prolonged hospital stay and decreased long-term survival[15, 16, 19].

SOS is caused by the toxic effect of oxaliplatin on hepatic sinusoidal endothelial cells (SECs)[20, 21]. The ensuing swelling of SECs and loss of sinusoidal wall integrity impairs sinusoidal blood flow, resulting in congestive obstruction. Eventually, peliosis, centrilobular hepatic vein fibrotic obstruction, perisinusoidal fibrosis and nodular regenerative hyperplasia (NRH) can occur[14].

The clinical symptoms as well as the histopathological findings suggest that SOS interferes with hepatic perfusion. Hypoperfusion might influence the tumour microenvironment and drug delivery to tumour cells. In line with this hypothesis, one would expect that patients developing SOS respond less well to chemotherapy in terms of tumour regression. The aim of the present study was to correlate the presence of SOS with TRG in patients with CLMs treated with oxaliplatin-based chemotherapy.

METHODS

Patients

Patients with CLMs who underwent a first partial hepatectomy at Maastricht University Medical Centre between January 2008 and December 2009 were included in the present study. Exclusion criteria were (a) no oxaliplatin-based neoadjuvant chemotherapy treatment, (b) hepatectomy specimen not containing a lesion or containing a non-neoplastic lesion (e.g. cavernous hemangioma), (c) inadequate histopathological material available (e.g. absence of non-tumour-bearing liver on a distance of more than 2 cm from the tumour), and (d) laparoscopic liver surgery. In general, the treatment schedule consisted of 3 to 6 cycles of oxaliplatin-based chemotherapy combined with bevacizumab prior to surgery. The actual number of treatment cycles differed per patient and was dependent on the radiographic response measured by the 2000 response criteria in solid tumours (RECIST)[22], the patient's physical condition and sustained side effects.

Histopathological examination

The partial hepatectomy specimens were sliced before fixation. After fixation in 4 per cent buffered formaldehyde, several tissue samples were taken from each tumour nodule, from the non-tumour-bearing liver (at a minimum distance of 2 cm from the lesion and avoiding the subcapsular region) and from the resection margins. The tissue samples were routinely embedded in paraffin and 4 µm sections were cut. Histological sections were examined by two experienced HPB pathologists. In case of discrepancy, the section was discussed at a dual head microscope and consensus was reached.

Assessment of sinusoidal injury

Morphological analysis was based on haematoxylin and eosin (H&E), reticulin and Trichrome Masson stains. Histopathological features containing vascular and parenchymal changes were scored according to the classification of Rubbia-Brandt and colleagues[14]. Based on these criteria, sinusoidal dilatation (grade 0 to 3), centrilobular vein and perisinusoidal fibrosis and steatosis (absent or present) were graded categorically.

Assessment of tumour regression grade

Morphological analysis of TRG was based on H&E stains. Each liver metastasis was scored for TRG, using the approach described by Mandard and colleagues for the assessment of tumour regression after preoperative chemoradiotherapy of esophageal carcinoma, which was modified for liver metastases[11]. Based on these criteria, TRG was graded as follows: grade 1 showed absence of histological identifiable residual tumour and extensive fibrosis; grade 2 was characterized by the presence of rare residual tumour cells scattered through the fibrosis; grade 3 involved a substantial amount of residual tumour

cells but fibrosis dominated; grade 4 showed residual tumour cells outgrowing fibrosis; and grade 5 was characterized by the absence of any tumour regression.

Statistical analysis

Data are given as mean or median dependent on the nature of the data. Pearson's chi-square test was used for categorical data. A multivariable logistic regression analysis was performed to correlate patient characteristics (age, sex, number of cycles chemotherapy) and the presence of SOS with TRG. For this analysis, we combined moderate (grade 2) and severe (grade 3) SOS lesions, resulting in 3 groups (no lesions, mild lesions and moderate-severe lesions). The TRG grade was divided in 2 groups, resulting in no or rare residual tumour cells (grade 1-2) versus a substantial amount of residual tumour cells (grade 3-5). For all tests, a p-value below 0.050 was considered to indicate statistical significance. Statistical analyses were performed using SPSS version 15.0 for Windows (SPSS Inc. Chicago, IL, USA).

RESULTS

Patients

Between January 2008 and December 2009, 82 patients with CLMs underwent a first partial hepatectomy at Maastricht University Medical Centre. After excluding patients without oxaliplatin-based chemotherapy treatment (n = 22), hepatectomy specimen without tumour (n = 5), inadequate histopathological material (n = 3), and patients undergoing laparoscopic partial hepatectomy (n = 2), 50 patients were available for analysis. Males and females were equally distributed with a mean age of 61 years (range 40-79). Mean number of oxaliplatin cycles was 6 (range 1-12).

Histopathological examination

Thirty-two out of 50 patients (64 per cent) showed SOS lesions of any grade. In more detail, 13 patients (26 per cent) had mild SOS lesions and 19 patients (38 per cent) had moderate or severe SOS lesions. Centrilobular vein and perisinusoidal fibrosis was present in 29 patients (58 per cent) and steatosis in 27 patients (54 per cent).

The TRG varied from grade 1 in 5 (10 per cent), grade 2 in 7 (14 per cent), grade 3 in 14 (28 per cent), grade 4 in 16 (32 per cent) and grade 5 in 8 patients (16 per cent), respectively. In patients with more than one metastasis, the TRG was similar for the different metastases.

Relation between SOS and TRG

A higher grade of SOS was associated with a higher TRG (p=0.016) with a regression coefficient of 1.416 (95 per cent confidence interval 0.265 – 2.567) (Table 9.1). TRG did not correlate with age, sex or number of cycles of oxaliplatin (Table 9.1). The presence of SOS was significantly correlated with hepatic fibrosis (p=0.003), but not with steatosis or number of treatment cycles (data not shown).

Table 9.1 Regression coefficients from multivariable logistic regression analysis of the effect of patient characteristics and SOS grade on tumour regression grade.

	Regression coefficient (95 per cent confidence interval)	**p-value**
Age	0.066 (–0.015 – 0.148)	0.111
Sex	–1.326 (–2.976 – 0.329)	0.116
Male (*n* = 25)		
Female (*n* = 25)		
SOS grade	1.416 (0.265 – 2.567)	0.016
0 (*n* = 18)		
1 (*n* = 13)		
2 and 3 (*n* = 19)		
***N* oxaliplatin cycles**	–1.121 (–2.813 – 0.565)	0.192
< 6 (*n* = 22)		
≥ 6 (*n* = 28)		

SOS, sinusoidal obstruction syndrome; *N*, number.

DISCUSSION

Oxaliplatin-based chemotherapy may affect the non-tumour-bearing liver, which leads to SOS. SOS is caused by toxic injury mainly targeting hepatic SECs, leading to a spectrum of lesions including sinusoidal dilatation, perisinusoidal fibrosis, NRH and peliosis[14]. Studies report that 22 to 74 per cent of patients receiving oxaliplatin-based neoadjuvant chemotherapy develop SOS of any grade[13-16, 23]. Our study is consistent with recent literature and shows a prevalence of sinusoidal lesions of 64 per cent, which is at the upper bound of reported rates. The relatively high incidence in our cohort might have three explanations, that is the fact that only oxaliplatin-based chemotherapy regimen were included, extensive sampling of the non-tumour-bearing liver was performed and specific attention to sinusoidal lesions was paid, as it was our main study endpoint.

We found that the presence of SOS was significantly associated with a higher TRG, which corresponds with a lower tumour response, to oxaliplatin-based chemotherapy. Several explanations for this finding could be considered, in which hepatic hypoperfusion and oxidative stress play important roles. First, clinical symptoms and pathological features suggest that SOS impedes optimal hepatic perfusion, which may induce a state

of hepatic hypoxia. Two recent studies reported hepatic upregulation of, among others, the expression of hypoxic factor (HIF-1α), hepatic fibrosis/hepatic stellate cell activation (COL3a1, COL3a2, PDGF-A, TIMP-1, and MMP-2), oxidative stress and angiogenic factors (VEGF-C) in oxaliplatin-related SOS relative to controls, suggesting that livers suffering from SOS are in a hypoxic state[24, 25]. Tumour hypoxia stimulates angiogenesis and cell signalling, in pathways such as c-met and lysyl oxidase-mediated signalling, which might promote tumour cell invasion[26]. Our observations indirectly support these findings by the negative correlation between SOS and tumour response. It would be clinically relevant to study polymorphisms in, for example, anti-oxidant genes that may identify subgroups of patients prone to develop SOS and, concomitantly, respond poorly to chemotherapy. A second explanation involves a reduced penetration of oxaliplatin at the tumour sites secondary to the obliterative sinusoidal lesions, which might lead to a reduced cytotoxic effect of oxaliplatin.

To the best of our knowledge, this is the first study that shows a significant association between the presence of SOS and higher TRG. Previous studies showed that TRG was important for the prediction of long-term outcome in patients with CLMs treated by chemotherapy and liver surgery[10, 12]. Rubbia-Brandt and colleagues found no significant correlation between SOS and TRG, though this was not the main focus of their paper[10]. As the present study was solely histopathologically based and had a short follow-up, the long-term survival data described in aforementioned papers could not be confirmed. A recent study by Tamandl and colleagues demonstrated that the presence of SOS was associated with early CLM recurrence, evidencing even more that SOS plays an important role in changing the tumour environment which may affect survival rates[19].

In summary, advanced stage SOS is associated with lower tumour response to oxaliplatin-based neoadjuvant chemotherapy in patients with CLMs. This may impede the likelihood of curative surgical resection and decrease long-term survival rates. We suggest that hypoperfusion and hypoxia are important factors, but further studies are needed to elucidate the exact underlying pathophysiological mechanisms.

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Chapter 10

The flavonoid monoHER prevents monocrotaline-induced hepatic sinusoidal injury in rats

Adapted from:

Ezzat T, van den Broek MA, Davies N, Dejong CH, Bast A, Malagó M, Dhar DK, Olde Damink SW. The flavonoid monoHER prevents monocrotaline-induced hepatic sinusoidal injury in rats. *J Surg Oncol* 2012; 106: 72-8.

ABSTRACT

Background

Sinusoidal obstruction syndrome (SOS) develops in 50 to 70 per cent of patients after oxaliplatin-based treatment for colorectal liver metastases. SOS is associated with portal hypertension and is caused by oxidative damage to endothelial cells and subsequent matrix metalloproteinase (MMP) induction. We studied the effect of the flavonoid monoHER on SOS prevention.

Methods

A monocrotaline (MCT) model was used to induce SOS in rats, with pre-treatment with monoHER. Portal pressure, hepatocellular damage, hepatic inflammation and MMP expression were studied *in vivo*. The potential inhibition of oxaliplatin-associated cytotoxicity by monoHER was tested *in vitro* in colorectal cancer cell lines.

Results

MonoHER ameliorated the increase in portal pressure after MCT (72hr: 7.3±2.7 versus 11.4±3.0 mmHg, p=0.016, MCT+monoHER versus MCT). MonoHER also prevented hepatocellular damage reflected by ALAT (72hr: 129.5±114.5 versus 311.9±163.6 IU/L, p=0.028, MCT+monoHER versus MCT). The histological liver damage score was lower in the monoHER group (72hr: 4.8±3.6 versus 10.3±0.5, p=0.002, MCT+monoHER versus MCT) concomitant with less inflammatory cell infiltration. Livers of MCT treated rats had higher expression of MMP-9 when compared to their MCT+monoHER counterparts at both 24hr (p=0.016) and 72hr (p<0.001). MonoHER had no effect on the *in vitro* proliferation of colorectal cancer cells when used either alone or in combination with oxaliplatin.

Conclusion

MonoHER prevented MCT-induced portal hypertension and hepatic injury in an experimental rat model. *In vitro* experiments showed no effect of monoHER on the cytotoxicity of oxaliplatin in colorectal cancer cells.

INTRODUCTION

Partial liver resection remains the only single modality to achieve curative treatment for colorectal liver metastases (CLMs), but unfortunately only 15 to 25 per cent of patients are initially eligible for resection[1]. The introduction of systemic chemotherapy regimens including irinotecan and oxaliplatin improved resectability rates[1, 2]. Oxaliplatin-based chemotherapy is related however, to the development of hepatic sinusoidal obstruction syndrome (SOS)[3]. Different SOS grades resulting from oxaliplatin develop in about 50 to 70 per cent of patients. SOS is associated with increased post-resectional morbidity secondary to portal hypertension and intra-operative hemorrhage, which results in a prolonged hospital stay[4-8]. Rare, fatal cases of SOS have been reported following oxaliplatin-based neoadjuvant chemotherapy[9, 10].

The pathophysiological mechanism of hepatic SOS has been well studied in the monocrotaline (MCT) rat model[11]. In short, monocrotaline or other toxic agents, such as oxaliplatin, cause damage to sinusoidal endothelial cells (SECs) by depletion of glutathione, which leads to depolymerization of F-actin. This, in turn, causes swelling of SECs and increased synthesis and activity of matrix metalloproteinases (MMPs), which break down the extracellular matrix. As a result, there is loss of SEC fenestration and formation of gaps between SECs allowing erythrocytes to penetrate into the space of Disse. The blood flow then dissects and separates the SEC lining, which subsequently blocks the sinusoids. Secondary to these events, an inflammatory reaction, characterized by accumulation of neutrophils in the sinusoidal region, occurs[12, 13].

Interventions to prevent the development of SOS focus on agents that either deactivate MMP activity, restore glutathione levels or preserve sinusoidal perfusion[14-16]. These agents have solely been tested in experimental models with mixed, but promising results and validation in clinical trials is pending. 7-Monohydroxyethylrutoside (monoHER) is a potential protective agent against SOS because of its favorable effect on the microvascular endothelium and its antioxidant and anti-inflammatory capacities[17]. In the past decade, our group has shown that monoHER provides a protective effect against doxorubicin-induced cardiotoxicity in mice without interfering with its anti-tumour activity[18, 19] and has tested it in phase I and II clinical trials in humans without any serious side effects[20, 21].

The aim of this study was to assess the protective effect of the flavonoid monoHER on the development of MCT-induced sinusoidal injury *in vivo* in rats. Furthermore, the effect of monoHER on cell growth of human colorectal cancer cells exposed to increasing concentrations of oxaliplatin was determined *in vitro*.

METHODS

Animals

This study was approved by the Animal Research Committee of University College London. All animal experiments were conducted according to Home Office guidelines under the UK Animals and Scientific Procedures Act 1986. Male Sprague-Dawley rats (Charles-Rivers, UK, *n* = 63), weighing 230–280 grams, were given free access to standard rodent chow and water, serially weighed, with a light/dark cycle of 12hr, at a temperature of 22–23°C and 50 per cent relative humidity.

Experimental design

The study consisted of 2 separate experiments, an *in vivo* study that investigated the effect of monoHER on portal pressure and hepatocellular damage in rats, and an *in vitro* experiment that studied the effect of monoHER + oxaliplatin on cell growth in human colorectal cancer cells. A rat model for SOS using a single gavage of MCT (MCT, Sigma Aldrich, St. Louis, MO, USA) was used for the *in vivo* study[11]. MonoHER was kindly provided by the Department of Pharmacology and Toxicology (Maastricht University Medical Centre, Maastricht, the Netherlands).

Study 1

Animals were divided into three groups; (Group 1) MCT+monoHER group (*n* = 21) received a single gavage of 160 mg/kg MCT in 0.5 ml dimethyl sulfoxide (DMSO) and 500 mg/kg monoHER dissolved in 36 mM NaOH (pH 7.8-8) intraperitoneally (i.p.) starting 1 day before MCT treatment and continued once daily up until sacrifice; (Group 2) MCT group (*n* = 21) received a single gavage of 160 mg/kg MCT in 0.5 ml DMSO and the vehicle of monoHER (0.5 ml of 36 mM NaOH) i.p. on the same time points as Group 1; and (Group 3) Sham group (*n* = 21), received a single gavage of the vehicle of MCT (0.5 ml DMSO) on day 1 and the vehicle of monoHER (0.5 ml of 36 mM NaOH) i.p. at the same time points as Group 1. Rats were studied at 24hr, 48hr and 72hr after MCT gavage (*n* = 7 at each time point).

Study 2

In order to examine the effect of MCT and monoHER on the cytotoxic effect of oxaliplatin in human colorectal cancer cells (LoVo and LS174T), increasing concentrations of oxaliplatin were added to these cells in combination with MCT and monoHER *in vitro*.

Study endpoints

Study 1

The primary endpoint was difference in portal pressure (PP) between the intervention and control group. Secondary endpoints were amount of liver damage assessed by alanine aminotransferase (ALAT) levels and histological examination, extent of sinusoidal injury assessed by electron microscopy, amount of hepatic inflammation, and MMP expression.

Study 2

The primary endpoint was cell viability measured by MTS-assay. The MTS-assay is a colorimetric assay that analyzes the activity of enzymes that reduce MTS to formazan, which allows assessing the viability and proliferation of cells in cell cultures.

Measurement of portal pressure

Rats were anaesthetized with inhalation of isoflurane. The body temperature was maintained at 37°C. A midline laparotomy was performed and the portal vein was cannulated with PE-5 tubing (VWR International, Leicestershire, UK) without disturbing the surrounding structures. Portal pressure (PP) was continuously monitored and recorded using a pressure transducer, a pressure amplifier and a computer equipped with a data recording and analysis system (BIOPAC systems, Norfolk, UK). The average pressure over a 5 min period was considered as the final portal pressure. Hereafter, blood samples were taken from the abdominal aorta and liver tissue was cut from the median lobe at sacrifice. A part of the liver tissue was preserved immediately in liquid nitrogen and the remaining portion in 10 per cent neutral formalin for downstream applications. Blood samples were drawn in pre-chilled tubes for EDTA-plasma, heparin-plasma and serum collection. Samples were stored at –80°C until determination.

Measurement of liver damage

Serum ALAT was measured using COBAS integra 400 biochemistry analyzer (Roche, Indianapolis, IN, USA). Paraffin sections were stained with haematoxylin and eosin (H&E) using standard histological techniques. Histopathological examination was performed in a blinded fashion by an experienced HPB pathologist. Liver damage was quantified using a modification of the scoring system described by Rubbia-Brandt and colleagues[4], including sinusoidal dilatation (graded on a 0 to 3 scale), perisinusoidal hemorrhage, peliosis, hepatocellular necrosis (graded on a 0 to 3 scale) and inflammatory cell infiltration (graded on a 0 to 3 scale). Mononuclear inflammatory cells were counted in three most densely populated areas at high power fields (HPF) (x400) and the average number of cells was considered as the number of cells/HPF.

Evaluation of MMP-2 and MMP-9 expression in tissue

Formalin-fixed tissue sections (5 µm thick) were used for immunohistochemistry. The immunohistochemical study was performed using the streptavidin ABC duet kit (Dako, Cambridgeshire, UK). Briefly, sections were dewaxed in xylene, rehydrated in graded alcohol and rinsed with PBS. Antigen retrieval was done by heating the sections in citrate buffer solution at 95°C for 10 min. Endogenous peroxidase activity was blocked by 3 per cent hydrogen peroxide for 20 min. The sections were incubated with the following primary antibodies: monoclonal rabbit anti-MMP-9 (1:200) (Abcam, Cambridge, UK) and monoclonal mouse anti-MMP-2 (1:200), (Abcam, Cambridge, UK). Streptavidin-peroxidase labelled secondary antibody was applied for 30 min at room temperature. Colour was developed with the chromagen 3,3'-diaminobenzidine (Dako, Cambridgeshire, UK) and counterstained with Mayer's haematoxylin solution. A scoring system based on both the intensity (graded on a 0 to 3 scale) and extent of staining (1 = <33 per cent, 2 = 33-67 per cent, 3 = >67 per cent) was used as a semi-quantitative measurement of MMP expression[22].

Evaluation of ultrastructural damage by electron microscopy

Perfusion fixation of the total liver in situ was performed according to the protocol that was detailed recently by our group[23].

In vitro effect of monoHER on human colorectal cancer cell growth

The effect of treatment with monoHER, MCT and oxaliplatin on colorectal cancer cell proliferation was assessed using the MTS-assay (Promega, Southampton, UK). Briefly, 2000 cells/well in a 96 well plate were incubated in RPMI-1640 media (Invitrogen, Paisley, UK) with increasing concentrations of (1) oxaliplatin 50-100-200 µM, (2) MCT 2-4-8 mM and (3) monoHER 25-50-100 mM or a combination of two of those. Following incubation for 48hr, 20 ul of MTS reagent was added in each well and incubated for another 2hr at 37°C in a CO_2 incubator. The absorbance was read at 490 nm using an LT-4000MS spectrophotometer (Labtech International, East Sussex, UK).

Statistical analysis

Data are given as mean with standard deviation. ANOVA with post-hoc test was used for multigroup analyses. This was performed using SPSS version 15.0 for Windows (SPSS Inc. Chicago, IL, USA). A p-value below 0.050 was considered statistically significant.

RESULTS

MonoHER reduced the portal pressure in SOS

MCT administration increased the PP compared to sham animals (Figure 10.1A). Pre-treatment with monoHER attenuated the increase in PP at all three time points (24hr: 7.4±0.9 versus 9.4±1.0 mmHg, p=0.005, 48hr: 7.9±1.3 versus 10.2±1.3 mmHg, p=0.019, and 72hr: 7.3±2.7 versus 11.4±3.0 mmHg, p=0.016, all MCT+monoHER versus MCT, respectively). There was no difference in PP between MCT+monoHER and sham animals (24hr: 7.4±0.9 versus 6.5±0.7 mmHg, p=0.227, 48hr: 7.9±1.3 versus 7.5±1.1 mmHg, p=0.783, and 72hr: 7.3±2.7 versus 6.6±0.5 mmHg, p=0.860, all MCT+monoHER versus sham, respectively).

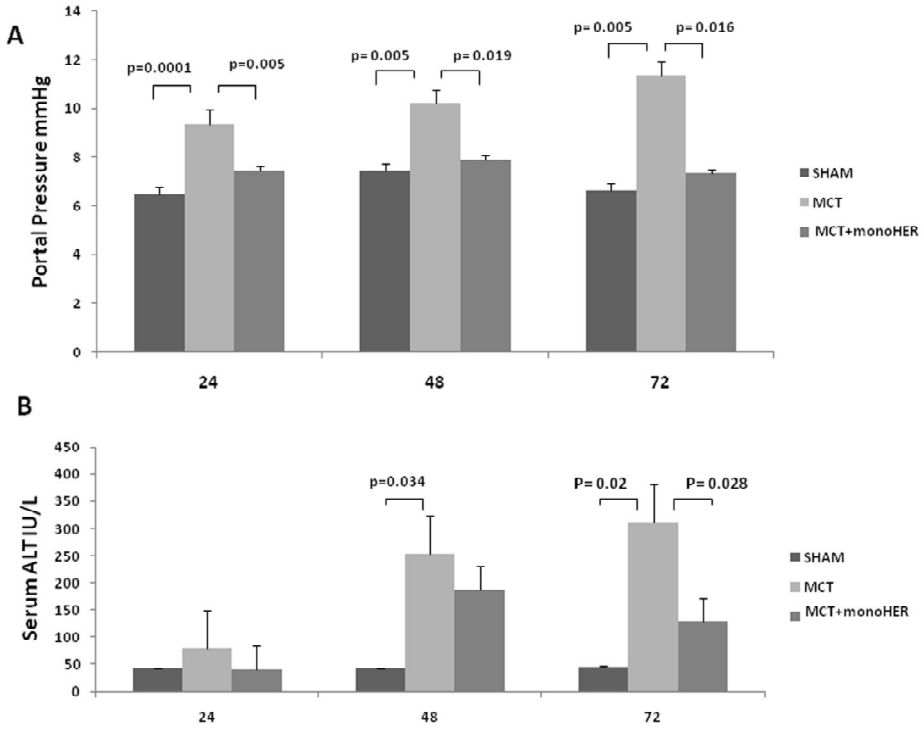

Figure 10.1A and 10.1B PP and serum ALAT measurements in the studied groups.
(A) MCT administration caused a significant rise in PP in the MCT group compared to the sham group. Pre-treatment with monoHER significantly reduced PP at all time points compared to the MCT group. **(B)** Rats pre-treated with monoHER had significantly reduced serum ALAT at 72hr compared to the MCT group. There was no difference between both groups at 24hr or 48hr. MCT rats had higher serum ALAT levels compared to sham rats at 48hr and 72hr, but not at 24hr.
Legend: MCT, monocrotaline; PP portal pressure; ALAT, alanine aminotransferase.

Liver damage was attenuated by monoHER

All animals survived the experiment. At sacrifice, the livers in the MCT treated rats appeared macroscopically congested at 24hr and had patchy necrotic areas at 48hr and 72hr. The macroscopic appearance of livers in the MCT+monoHER group was less congested at 48hr and 72hr (Figure 10.2).

Serum ALAT levels were higher in the MCT treated rats compared to sham rats at 48hr and 72hr (48hr: 253.4±171.7 versus 42.2±3.1 IU/L, p=0.034, and 72hr: 311.9±163.6 versus 46.2±4.3 IU/L, p=0.002, MCT versus sham, respectively). MCT+monoHER rats had lower ALAT concentrations when compared to MCT rats at 72hr (129.5±114.5 versus 311.9±163.6 IU/L, p=0.028). There was no difference in serum ALAT between MCT+monoHER and MCT rats at 24hr (p=0.092) and 48hr (p=1.000) (Figure 10.1B).

MCT administration resulted in extensive sinusoidal dilatation on histological examination, whereas a much lesser extent of dilatation was noticed in the MCT+monoHER group at 24hr (Figure 10.3A and 10.3B). At 48hr, marked congestion developed in the MCT as well as the MCT+monoHER groups with a much more extensive infiltration of inflammatory cells in the MCT compared to the MCT+monoHER group (Figure 10.3C and 10.3D). Bridging hepatic necrosis across the hepatic lobules along with

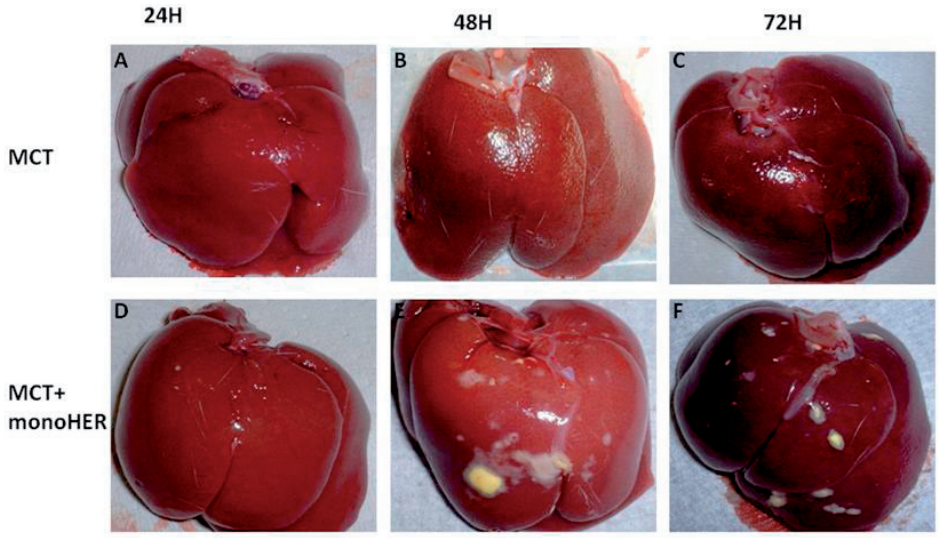

Figure 10.2A-F Representative samples showing the macroscopic appearance of the liver at different time points.
(A-C) The congestion is evident in the MCT group at 24hr, with appearance of punctuate hemorrhagic spots at 48hr and areas of frank necrosis in a deeply congested liver at 72hr, which resembles the blue liver seen in the clinical scenario. **(D-F)** Congestion also occurred in the MCT+monoHER group, however much less severe than in the MCT group and without development of punctuate hemorrhage or necrosis. Of important notice, the yellow color on the liver represents monoHER and not necrosis.
Legend: MCT, monocrotaline.

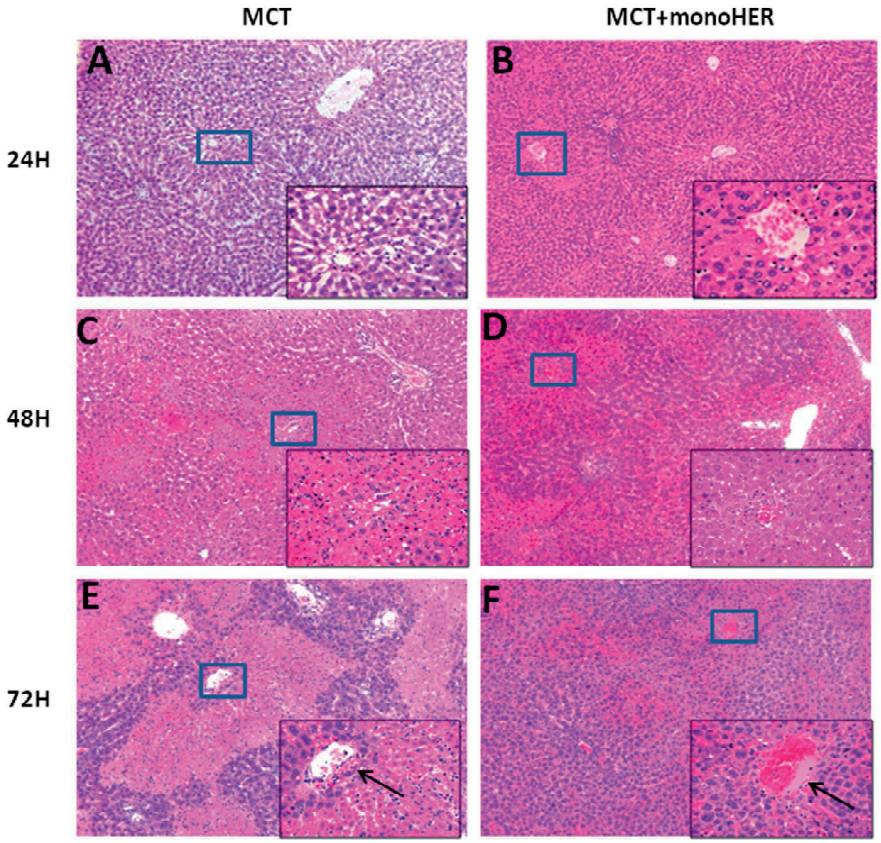

Figure 10.3A-F Representative photomicrographs of liver damage on histological level in the studied groups.
(A-B) There was more severe sinusoidal dilatation in the MCT group at 24hr compared to the MCT+monoHER group. **(C-D)** At 48hr, the sinusoidal dilatation was associated with hepatocellular damage mainly in the centrilobular zones in both groups, however with less inflammatory cells in the MCT+monoHER group. **(E-F)** At 72hr, there was massive necrosis all over the liver in the MCT group with marked inflammation with disruption of the SEC lining and extravasation of erythrocytes and inflammatory cells in direct contact with the hepatocytes. Necrosis did not develop in livers of MCT+monoHER rats and the integrity of sinusoidal cell lining was maintained in this group.
Legend: MCT, monocrotaline; SEC, sinusoidal endothelial cells; original magnification x40, inset x200.

dense inflammatory cell infiltration was observed in the MCT group at 72hr. Also, SEC detachment from the underlying basement membrane with evidence of subendothelial hemorrhage was observed in the MCT group (Figure 10.3E). In contrast, in the MCT+monoHER group, congestion was still present; however, hepatocellular necrosis and endothelial cell detachment were minimal (Figure 10.3F). The overall liver damage severity score was lower in the MCT+monoHER group at 72hr (4.8±3.6 versus 10.3±0.5, MCT+monoHER versus MCT, p=0.002) (Figure 10.4A). Inflammatory cell infiltration/HPF

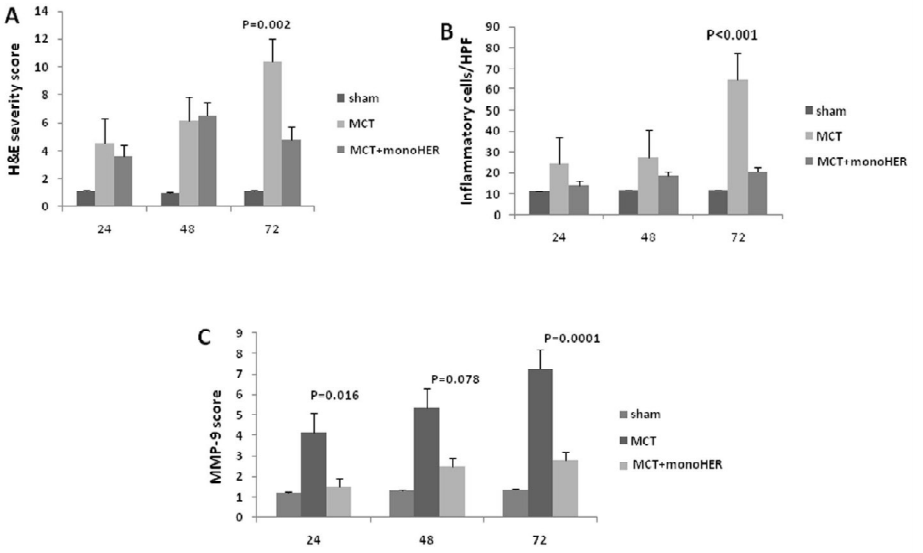

Figure 10.4A-C Semi-quantification of liver damage and MMP activity.
(A-B) The overall liver damage severity score and inflammatory cell count/HPF was significantly lower in the MCT+monoHER group at 72hr only compared to the MCT group. **(C)** MMP-9 expression was significantly lower in the MCT+monoHER group at 24hr and 72hr compared to the MCT group.
Legend: HPF, high power field; MCT, monocrotaline; MMP, matrix metalloproteinase.

was also lower in the MCT+monoHER group at 72hr (20.6±8.3 versus 64.5±9.0 cells/HPF, MCT+monoHER versus MCT, p<0.001), but not at any other time point (Figure 10.4B).

Ultrastructural damage was attenuated after monoHER

On scanning electron microscopy (SEM), large gaps were noticed in the sinusoids of the MCT group with less gap formation in the MCT+monoHER group. On transmission electron microscopy (TEM), gapping of the fenestrae was evident in the MCT group with detachment of the SEC lining from the basement membrane and disruption of the space of Disse. There was a slight degree of SEC separation that could be detected in the MCT+monoHER group without disruption of the space of Disse (Figure 10.5).

MonoHER treatment reduced MMP-9 expression in the liver

Livers of MCT treated rats had higher expression of MMP-9 when compared to MCT+monoHER rats at both 24hr (p=0.016) and 72hr (p<0.001) (Figure 10.4C). Areas of highest expression included SEC lining and to lesser extent inflammatory cells in the MCT group with less expression in the MCT+monoHER group (Figure 10.6A-D). In addition, MMP-9 expression was noticed in areas surrounding the hepatocellular necrosis at 72 hr which was not present in the MCT+monoHER group (Figure 10.6E and 10.6F). In

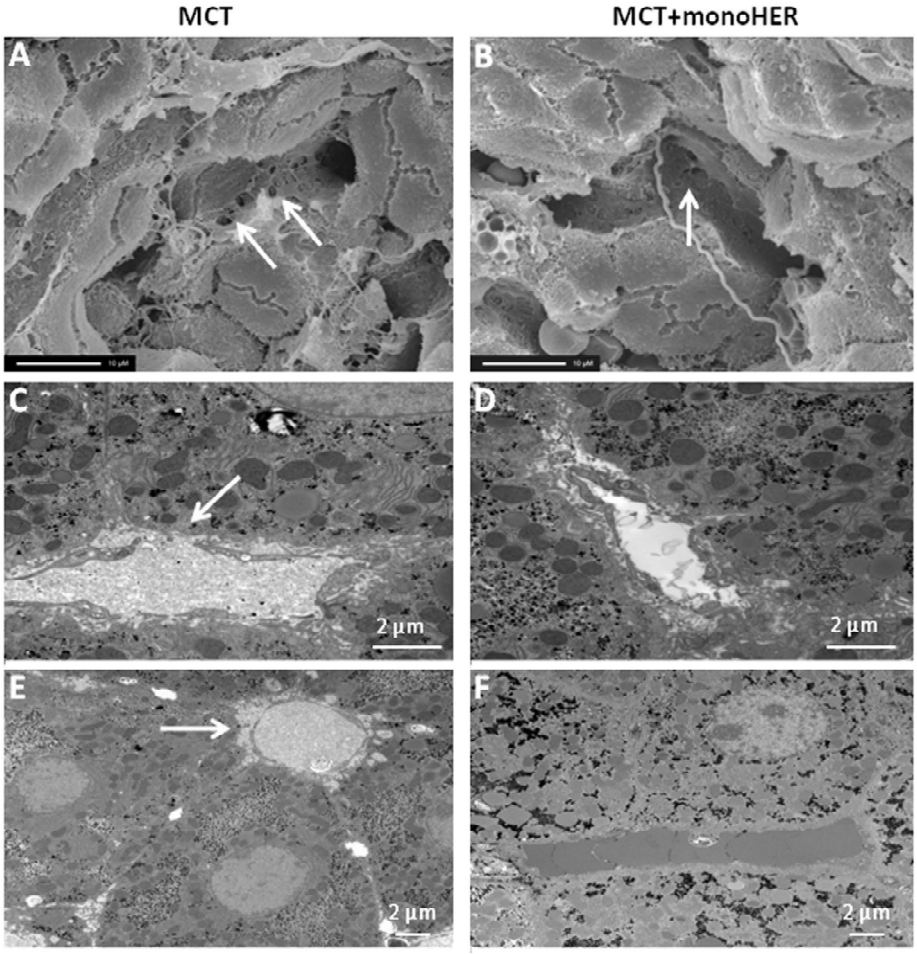

Figure 10.5A-F Electron microscopy of the liver at 72hr.
(A-B) Treatment with MCT induced damage at the structural level in the form of gap formation in the liver sinusoids with damage still present in the monoHER group, however with fewer gaps as seen on SEM. **(C)** TEM studies showed that there was gapping of the sinusoids in the MCT group leading to an increase in the size of fenestrae from the normal size of about 150 nm to about 200 nm. **(E)** Detachment of the SEC lining from the basement membrane was detected in the MCT group. **(D-F)** In the MCT+monoHER group, the integrity of the SEC lining was maintained with slight separation from the basement membrane but no disruption of the space of Disse.
Legend: SEM, scanning electron microscopy; TEM, transmission electron microscopy; SEC, sinusoidal endothelial cell; MCT, monocrotaline.

contrast to MMP-9 expression, MMP-2 expression was very weak in liver sections and no obvious differences could be detected among the three groups.

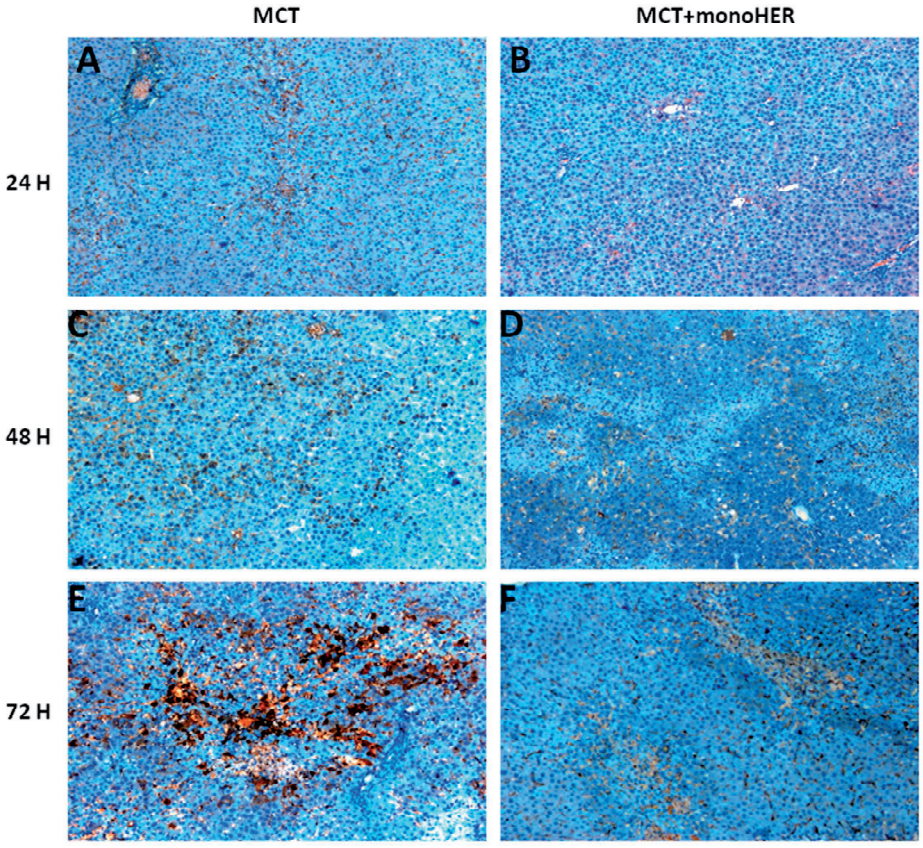

Figure 10.6A-F MMP-9 expression in the liver.
(A-B) MMP-9 was expressed as early as after 24hr in MCT group in the SEC lining, mainly in the centrilobular regions with less expression in the MCT+monoHER group. **(C-F)** An increase in expression was evident at 48hr and 72hr in the MCT group with less expression in the MCT+monoHER group.
Legend: MMP, matrix metalloproteinase; MCT, monocrotaline; original magnification x40.

MonoHER did not interfere with oxaliplatin cytotoxicity

Both colorectal cancer cell lines showed dose-dependent cytotoxicity for increasing doses of oxaliplatin. MCT and/or monoHER at different concentrations did not have any significant effect on cell numbers in both cell lines. Addition of monoHER to oxaliplatin, even in high dose (100 μM), did not interfere with the cytotoxic effect of oxaliplatin (Figure 10.7A and 10.7B).

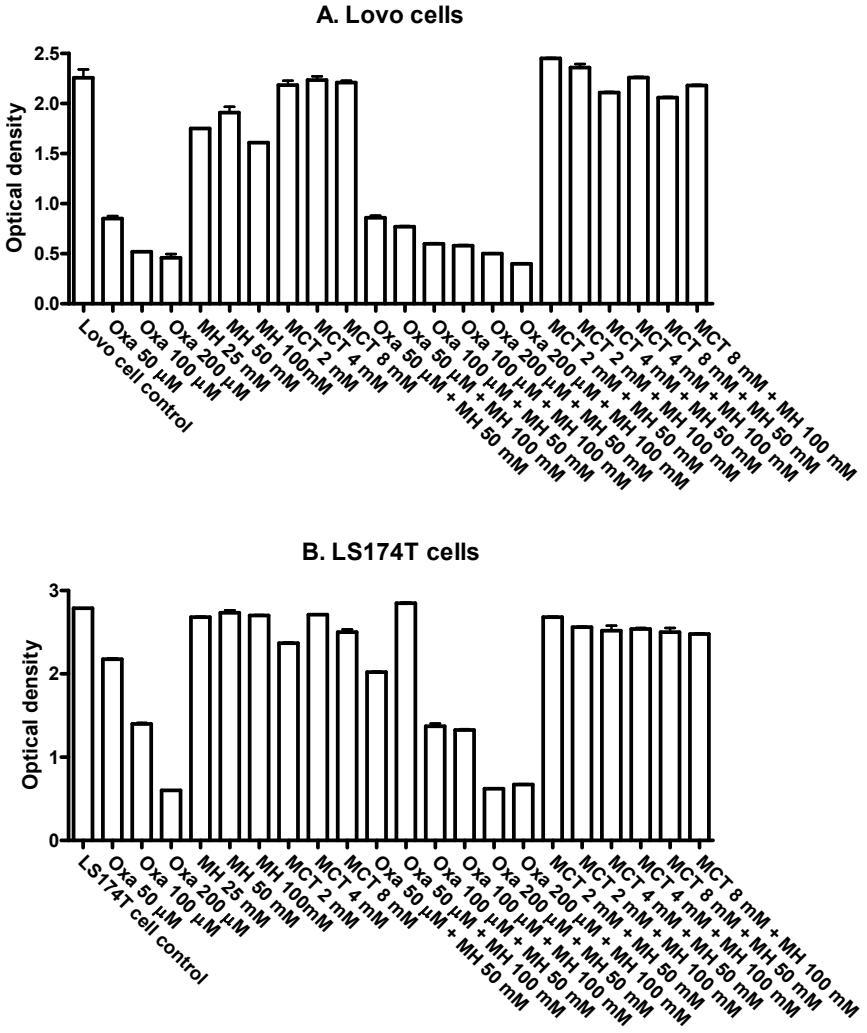

Figure 10.7A and 10.7B *In vitro* analysis of proliferation of colorectal cancer cell lines.
(A-B) MonoHER did not interfere with the dose-dependent cytotoxic effects of oxaliplatin. MCT and monoHER alone had no effect on viable cell count in either cell line (A: Lovo cells and B: LS174T cells).
Legend: oxa, oxaliplatin; MH, monoHER; MCT, monocrotaline.

DISCUSSION

The present study clearly demonstrated that monoHER can be used to prevent the development of portal hypertension and hepatocellular damage in an experimental model of SOS. Initially, we tried to establish a rat model of oxaliplatin-induced SOS, but this attempt was not successful. In a series of preliminary experiments, the maximum tolerable dose of oxaliplatin in rats (10 and 20 mg/kg i.p., respectively) was used. Oxali-

platin treatment induced only mild congestion without any significant hepatocellular necrosis, which is one of the main pathognomonic features of SOS[11]. The actual reason is unknown and might be related to an altered metabolism of oxaliplatin in particular species such as rats. These findings compelled us to use the reproducible MCT model for induction of SOS[11].

We found that the PP in the MCT+monoHER group was significantly lower compared to the MCT group at all time points. This was also reflected in the gross macroscopic appearance of the liver in the MCT+monoHER group, which demonstrated less severe liver congestion compared to the MCT group, specifically at 72hr. Similarly, monoHER prevented hepatocellular damage reflected by lower aminotransferase levels, less histological liver damage and less inflammatory cell infiltration. These observations could be explained by the pharmacological properties of monoHER, which can act as (1) endothelial cell stabilizer, (2) potent anti-oxidant and (3) anti-inflammatory compound. These capacities make monoHER a potential drug for the prevention of SOS as endothelial damage, oxidative stress and inflammation all are involved in its pathogenesis[24].

First, swelling of SECs followed by their detachment is key in the development of SOS and portal hypertension[13]. MonoHER is one of the constituents of Venoruton®, a drug that has been shown to protect the microvascular endothelium, as evidenced by a reduced number of circulating endothelial cells in patients with chronic venous insufficiency[25, 26]. The subendothelial localization of monoHER is thought to be partially responsible for these positive protective effects on the endothelium[27].

Secondly, it is well known that both MCT and oxaliplatin induce SOS by formation of reactive oxygen species secondary to glutathione depletion in SECs[11, 28]. Flavonoids such as monoHER have been shown to possess potent free radical scavenging properties and possibly, these anti-oxidant capacities played a role in prevention of SOS by monoHER through sweeping free oxygen radicals[29]. Earlier observations by DeLeve and colleagues showed, however, that SOS could be prevented by intraportal glutathione infusion, but not by infusion of other potent free radical scavengers[16].

Third, a recent study on the pathogenesis of SOS showed that acute phase pathway genes were upregulated in liver tissue following oxaliplatin therapy[30]. In the present study, monoHER played a pivotal role in reducing cell damage and inflammation secondary to MCT treatment, as evidenced by less structural liver damage, significantly lower serum aminotransferase levels and reduced number of inflammatory cells in the MCT+monoHER group. The reduced number of inflammatory cells could either be attributable to less damage and therefore a reduced stimulus for an inflammatory response or direct prevention of adhesion of neutrophils to sinusoidal endothelial cells in the liver. Indeed, we showed previously that monoHER attenuated doxorubicin-induced inflammatory reactions by inhibition of neutrophil adhesion to endothelial cells through down-regulation of VCAM and E-selectin[31]. Narita and colleagues also demonstrated

that inhibition of neutrophil adhesion to the sinusoids is the key factor in prevention of SOS following MCT intake in rats[12]. On the contrary, Hanumegowda and colleagues undermined the role of polymorphonuclear cells (PMNs) in MCT-induced SOS by showing PMN infiltration predominantly away from the centrilobular areas, which represent the most heavily affected areas of sinusoidal damage[32]. In addition, PMN depletion did not prevent the sinusoidal damage. Although PMNs might not apparently play a significant role in sinusoidal damage, they definitely play an integral role in hepatocellular damage in several ways including release of superoxide radicals and activated MMP-9[32].

The present study also showed a role of monoHER in interfering with another important downstream pathway in SOS development, which is MMP-9 expression. The main sources of MMP-9 expression are PMNs and SECs[32-34]. The MCT pyrrole, which is the active metabolite of MCT, interacts with F-actin in SECs leading to F-actin depolymerization and swelling of SECs. F-actin depolymerization is linked to increased MMP activity in the extracellular matrix. The upregulation of MMPs leads to degradation of basement membrane components and subsequent SEC detachment[13]. DeLeve and colleagues noticed high expression of MMP-9 and to a lesser extent MMP-2 in the basement membrane of SECs after MCT and concluded that this might be responsible for separation and dissection of the SEC from the basement membrane[15]. In the present study, MMP-9 expression was restricted predominantly in the SEC lining in the first 24 to 48hr, however, at 72hr, expression extended to areas of hepatocellular damage with significantly less expression in the MCT+monoHER group. It can be hypothesized that monoHER plays a pivotal role in the stabilization of the basement membrane by diminished MMP-9 expression due to maintenance of SEC integrity or diminished inflammatory cell influx.

The advantage of monoHER lies within its effectiveness in preventing SOS without interference with the cytotoxic activity of oxaliplatin. MonoHER itself had no effect on the growth rate of colorectal cancer cells and, when used along the cytotoxic doses of oxaliplatin, it did not interfere with the anti-proliferative effects of oxaliplatin. This is in agreement with our previous results, where monoHER achieved adequate intracellular concentration without interfering with the pharmacokinetics of doxorubicin[18, 35]. In addition, monoHER has been proven to be safe in a phase I clinical trial and has been tested in a phase II clinical trial in man[20, 21]. Recently, concerns have been raised towards the oxidation product of monoHER, which may be toxic[29]. Similar to quercetin, the oxidation product of monoHER reacts *in vitro* and *in vivo* with the thiol group of glutathione, possibly leading to its depletion. This would lead to catastrophic consequences following monoHER therapy since the primary insult in SOS is glutathione depletion. Fortunately, this has not been shown to occur in patients owing to a structural difference between monoHER and quercetin. Unlike quercetin, which easily reacts with protein thiols, oxidized monoHER is reduced by its preferential binding with plasma ascorbate, hence the risk of glutathione depletion is minimal[36].

To conclude, monoHER is a potential therapeutic agent to be used in prevention of oxaliplatin-induced SOS owing to its potent vasoprotective, anti-oxidant and anti-inflammatory effects ameliorating portal hypertension, sinusoidal injury and hepatocellular damage in an experimental SOS rat model.

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Part IV

Summary and future perspectives

Chapter 11

Summary and general discussion

There has been substantial progress in the field of liver surgery owing to improved patient selection, novel surgical techniques and optimization of perioperative care[1]. As a result, morbidity and mortality rates after partial liver resection have gradually declined[2, 3]. Due to the enhanced safety, the indications for partial hepatectomy have broadened towards more extensive resections in high-risk patient groups. The resultant small remnant liver volume and impaired preoperative liver function pose a risk of post-resectional liver failure (PLF). Although the incidence of PLF is relatively low, it may have deleterious consequences and is associated with high mortality rates (**chapter 1**). Risk factors for PLF are either surgery or patient-related. As curative treatment options are lacking to date, prevention of PLF is of vital importance to improve outcomes of liver surgery.

The broader aim of this thesis was to assess and improve outcomes of patients undergoing liver surgery. This then translated into three more specific aims looking at (1) the feasibility of randomized controlled trials (RCTs) in liver surgery using clinical endpoints, (2) surgery-related risk factors of PLF and (3) patient-related risk factors of PLF. Taken together, the aims of the present thesis were as follows:

Aim 1: to develop strategies that increase the feasibility of conducting RCTs in liver surgery using clinical outcomes as primary endpoints (chapter 2, 3 and 4).

Aim 2: to evaluate the effect of two surgical techniques on hepatic damage in patients undergoing liver surgery (chapter 5 and 6).

Aim 3: to study the effect of oxaliplatin-based chemotherapy on hepatic damage in patients undergoing partial liver resection for colorectal liver metastases (CLMs) (chapter 7, 8, 9 and 10).

PART I. ASSESSING OUTCOMES OF LIVER SURGERY: CRITICAL APPRAISAL OF CLINICAL ENDPOINTS

The first aim of this thesis was to develop strategies that increase the feasibility of performing RCTs in liver surgery using clinical endpoints.

In the current era of evidence-based surgical practice, treatment recommendations are preferentially based on high level of evidence research, such as RCTs or meta-analyses. The performance of RCTs in the field of liver surgery is challenging, which may explain the low number of randomized studies performed in this area to date[4, 5]. The challenges are mainly related to practical issues, such as difficulty of recruiting a sufficient number of patients in a timely manner, and design issues, such as the selection of a suitable primary endpoint that accurately reflects the effect of the intervention[6]. Traditionally, short-term clinical endpoints such as operative mortality and total morbidity are used for the evaluation of surgical interventions[7]. Therefore, part I of this thesis focuses on

facilitating the conduct of RCTs using clinical outcomes of liver surgery as primary endpoints.

In **chapter 2**, the feasibility of conducting an RCT in liver surgery using a single component, clinical outcome as primary endpoint was evaluated. A systematic literature review was performed to estimate the mean incidence rate of surgery-related mortality and specific types of morbidity in current hepatobiliary (HPB) practice. Mean operative mortality rate was 1.0 per cent and mean total morbidity rate 28.9 per cent; mean rates of bile leakage and post-resectional liver failure were 4.4 and 2.6 per cent, respectively. Based on these mean incidence rates, the smallest numbers of patients needed in each arm of an RCT using a clinical outcome as primary endpoint were calculated. As a rule of thumb, the sample size of a trial is negatively correlated to the incidence rate of the outcome parameter under study[8]. Consequently, the computed sample sizes turned out to be extremely large. An RCT aiming to show a one-third relative reduction in operative mortality had to include 15 614 patients in each arm. The same RCT aiming to show a one-third relative reduction in PLF had to include 5 924 patients in each arm. As approximately 500 liver resections are performed annually in the Netherlands and approximately 7000 in the United States, we concluded that the feasibility of conducting an adequately powered liver surgery-related RTC using a single component, clinical outcome as primary endpoint was low. We postulated that strategies facilitating the conduct of high level of evidence research in liver surgery using clinical outcomes as primary endpoints include the use of composite endpoints (CEPs), organization of multicentre trials and/or performance of meta-analyses. Alternatively, liver surgery-related surrogate endpoints can be adopted as primary endpoints, but robust validation of the latter is lacking[9].

In order to facilitate the use of CEPs and conduct of meta-analyses, uniform definitions of complications of liver surgery are needed[10]. The use of standard definitions reduces inconsistencies in trial reporting, allows for unequivocal interpretation of trial data and facilitates comparison of trial results[7]. In **chapter 3**, standard definitions of clinical outcomes of liver surgery were proposed. Results of a systematic literature review showed that clear definitions of clinical endpoints of RCTs in liver surgery were only provided in less than one-third of trial reports, and, if present, differed substantially. A web-based survey among 54 international experts in HPB surgery was undertaken in order to reach consensus on definitions of the most frequently used clinical endpoints of RCTs in liver surgery. The survey had a response rate of 57 per cent. Based on the comments of the respondents, the proposed definitions were adjusted. These final definitions are open to discussion and need to be validated in large, prospective patient cohorts.

In advance of formal validation, the proposed definitions were used for the design of a liver surgery specific CEP in **chapter 4**. A CEP is a combination of two or more procedure specific outcomes that are considered as a single, dichotomous endpoint[11].

CEPs lead to an increased statistical power because their event rate is higher than that of a single component, clinical endpoint. As a consequence, the sample size needed for an adequately powered RCT decreases likewise. Based on an electronic survey among international experts in HPB surgery, a consensus-based and well-defined liver surgery specific CEP was developed which included ascites, PLF, bile leakage, intra-abdominal hemorrhage, intra-abdominal abscess, and operative mortality, all occurring within 90 days of initial liver surgery. Caution should be exercised when interpreting CEPs, as they can be misleading if components vary widely in clinical relevance or incidence[12, 13]. Therefore, all components of the liver surgery specific CEP had to be classified as Clavien-Dindo severity grade of at least 3[14]. In addition, the incidence rate of the liver surgery specific CEP and its components was evaluated in 815 patients who had undergone liver surgery in two high-volume European HPB centres. The incidence of the individual components ranged between 1 and 10 percent. The incidence of the liver surgery specific CEP was 11 per cent in one cohort and 19 per cent in the other. These rates led to an approximately twofold reduction in the sample size required for an adequately powered RCT using the liver surgery specific CEP instead of a single component, clinical endpoint as primary endpoint. The clinical utility of the proposed liver surgery specific CEP seems promising, however, prospective evaluation is warranted. Moreover, it may be necessary to design CEPs customized for medication or surgical technique trials, to assign weight to its components or to develop CEPs that include long-term outcomes of liver surgery[15].

In conclusion, the results of part I of this thesis showed that the feasibility of conducting adequately powered RCTs in liver surgery using surgery-related mortality or morbidity as primary endpoint was low, as such endpoints require large sample sizes owing to their low incidence. The implementation of a consensus-based and well-defined liver surgery specific CEP consisting of ascites, PLF, bile leakage, intra-abdominal hemorrhage, intra-abdominal abscess, and operative mortality was able to overcome this problem. Prospective validation of the latter CEP is warranted.

PART II. ASSESSING OUTCOMES OF LIVER SURGERY: HEPATIC DAMAGE AS A RESULT OF SURGICAL TECHNIQUE

The second aim of the present thesis was to evaluate the effect of different surgical techniques on hepatocellular damage in patients undergoing liver surgery.

Intra-operative blood loss and the need for blood transfusions are related to adverse short- and long-term outcomes of liver surgery[16-18]. Procedures to limit blood loss include mobilization of the liver and intermittent occlusion of the hepatoduodenal ligament (i.e. Pringle maneuver)[19]. The rationale for their use is under discussion as both procedures induce profound hepatocellular damage[20-22]. In order to examine this liver cell damage

in more detail, these procedures were separately assessed in two studies in humans described in part II of the present thesis.

During mobilization, the liver is forcefully manipulated in order to dissect its ligaments and control direct venous branches to the inferior caval vein. Our group previously showed that manipulation of the liver was the leading cause of surgery-induced liver cell damage[23]. In addition, we observed that manipulation of the liver during liver surgery was followed by a systemic inflammatory response. Animal models suggest that activation of hepatic immune cells plays a central role in the development of mobilization-induced liver injury and subsequent clinical outcome, but these results need confirmation in man[24]. It is important to unravel the link between mobilization-induced liver cell damage and hepatic inflammation, as it is possible to modulate this inflammatory response. In **chapter 5**, the association between liver mobilization, hepatocellular damage and hepatic inflammation was established in humans. We demonstrated that liver mobilization was associated with hepatocellular damage and hepatic inflammation, as shown by hepatic infiltration of MPO and CD68-positive inflammatory cells and hepatic upregulation of mRNA of pro-inflammatory cytokines, such as interleukin (IL) 1β, IL-6 and IL-8. In addition, there was a significant correlation between the duration of mobilization and the increase in MPO-positive cells after mobilization. The relation between manipulation-induced liver injury and post-resectional outcomes could not be established due to the relatively small patient numbers in the present study. The question therefore still remains whether the observed hepatic inflammatory response is beneficial or not. Based on aforementioned experimental data, we hypothesize that prevention of mobilization-induced liver cell damage and inflammation could be of clinical importance. Preventive strategies include either employment of alternative surgical techniques, such as the anterior approach or laparoscopic surgery, or modulation of the inflammatory response[21, 25-27]. In order to identify subgroups that would benefit most from these preventive measures, studying differences in mobilization-induced hepatocellular damage between livers without and with underlying disease, such as chemotherapy-associated hepatotoxicity or steatohepatitis, would be relevant.

Transection of liver parenchyma usually follows mobilization of the liver. In order to limit blood loss during transection, an intermittent Pringle maneuver (IPM) may be applied. The optimal duration of the ischemic intervals during IPM is unknown and depends on the balance between ischemia-induced hepatocellular damage and blood loss, both resulting in postoperative morbidity[28]. Two recent RCTs showed that IPM using 30 min ischemic intervals resulted in similar remnant liver function and hepatocellular damage compared to IPM using 15 min ischemic intervals, while intra-operative blood loss was lower in the 30 min ischemic interval groups[29, 30]. However, up until now, consequences of different ischemic intervals were assessed using aminotransferase levels on different postoperative days. It remains uncertain if the assay of aminotransferases is sufficiently

sensitive to detect small differences in hepatocellular injury[31]. Intra-operative monitoring of liver fatty acid-binding protein (L-FABP) plasma levels may be a more direct and sensitive method to analyze liver cell damage. L-FABP is a cytosolic protein with a small molecular mass, which is involved in intracellular fatty acid transport and lipoprotein metabolism[32, 33]. After an insult to the liver, a rapid rise in plasma L-FABP is observed as result of a quick release of intracellular L-FABP from damaged, dying hepatocytes[34]. In **chapter 6**, we showed in a randomized study in patients undergoing liver surgery that IPM using 30 min ischemic intervals resulted in similar hepatocellular damage, as reflected by L-FABP, compared to IPM using 15 min ischemic intervals. Moreover, remnant liver function was preserved after prolonged IPM. By measuring L-FABP fluxes over the gut, we did not find evidence for the induction of intestinal damage secondary to inflow occlusion and portovenous stasis. A follow-up study of our group, however, showed that IPM was associated with intestinal epithelial damage, reflected by increased intestinal fatty acid-binding protein levels, and gut barrier dysfunction, evidenced by endotoxemia[35]. Of important notice, no IPM led to significantly less hepatocellular damage in the present RCT. As a recent meta-analysis failed to show a clinical advantage of IPM, we recommend not to use intermittent pedicle clamping during liver surgery[20]. If IPM is used, 30 min ischemic intervals are preferred for safe liver surgery.

In conclusion, we demonstrated in part II of the present thesis that mobilization of the liver was associated with liver cell damage and hepatic inflammation proportional to its duration. Modulation of this inflammatory response might be beneficial, although data in man are lacking. IPM using 15 or 30 minutes ischemic intervals induced comparable hepatocellular damage, reflected by systemic L-FABP levels, whereas no IPM resulted in significantly less liver cell injury. Therefore, IPM should only be used when required for safe liver surgery.

PART III. ASSESSING OUTCOMES OF LIVER SURGERY: HEPATIC DAMAGE AS A RESULT OF OXALIPLATIN-BASED CHEMOTHERAPY

The third aim of this thesis was to study hepatic sinusoidal injury secondary to oxaliplatin-based chemotherapy in patients undergoing partial liver resection for CLMs.

Liver surgery is the only treatment with the potential of long-term cure for patients with CLMs[36]. Unfortunately, only a minority of patients with CLMs present with initially resectable disease. Therefore, a multidisciplinary approach consisting of neoadjuvant chemotherapy followed by surgery has become standard of care. Perioperative chemotherapy treatment has proven to be able to downsize tumour mass, enhance resectability rates, and increase progression-free survival after liver surgery for CLMs[37, 38]. However, oxaliplatin-based chemotherapy is associated with injury to hepatic sinusoidal

endothelial cells (SECs) of the non-tumour-bearing liver, which may manifest as sinusoidal obstruction syndrome (SOS)[39-41]. The presence of sinusoidal lesions is related to a decreased regenerative capacity and enhanced post-resectional morbidity after major liver resection, such as PLF and PLF-related death[41-43]. As the presence of SOS is a risk factor of PLF, we explored the clinical consequences of sinusoidal injury, analyzed a non-invasive marker for its detection and assessed a potential protective agent.

Chapter 7 illustrated the clinical problem of severe sinusoidal injury of the non-tumour-bearing liver in two patients who had undergone major liver surgery for CLMs. Both patients had no history of pre-existent liver disease and were treated with 6 cycles of oxaliplatin-based chemotherapy prior to liver surgery. Histopathological analysis of the resection specimen showed severe sinusoidal injury of the liver consistent with sinusoidal congestion and nodular regenerative hyperplasia (NRH). Along with the presence of NRH, portal hypertension developed postoperatively which led to life-threatening complications and death of one patient. Although the present report describes only two cases, the presence of sinusoidal lesions after oxaliplatin-based chemotherapy for CLMs is a frequent clinical problem with lesions of any grade reported in 22 to 74 per cent and NRH in 25 per cent of patients[40, 41, 44-46]. Given the toxic effects of oxaliplatin-based chemotherapy on the non-tumour-bearing liver, it seems advisable to limit its administration *prior* to surgery. If oxaliplatin-based neoadjuvant chemotherapy is necessary to achieve liver surgery with curative intent, preoperative detection of SOS and institution of preventive measures are important.

Information on the presence of sinusoidal injury prior to major liver surgery is clinically relevant, as it may alter the surgical strategy. As patients with SOS have a decreased functional reserve, they may benefit from restrictive surgery or two-stage procedures. The gold standard for the detection of SOS is measurement of the wedged hepatic venous pressure gradient and assessment of liver pathology[47]. The drawbacks of these invasive procedures are well known, and therefore, non-invasive detection methods have been developed. These methods use indirect markers of sinusoidal injury and have therefore modest sensitivity and specificity for the detection of SOS[41, 45]. In **chapter 8**, the hypothesis that systemic hyaluronic acid (HA) level would be an accurate marker of SOS secondary to oxaliplatin-based chemotherapy in patients with CLMs was explored. HA is synthesized throughout the body by cells of mesodermal lineage. After the release of HA into the systemic circulation, it is rapidly cleared by a receptor-facilitated removal mechanism almost solely present in hepatic SECs[48, 49]. Therefore, HA is regarded as a marker of hepatic SEC function. As SEC damage is a prime event in the development of SOS, we hypothesized that HA levels would differ between patients with and without SOS. In our cohort, SOS lesions of any grade were present in 58 per cent of patients treated with oxaliplatin-based chemotherapy. Systemic HA levels were significantly higher in patients with moderate or severe SOS compared to patients with no or mild

SOS, with a fair diagnostic performance for detection of advanced SOS. The optimal cutoff value of systemic HA levels of 44.1 ng/mL yielded a sensitivity of 67 per cent and specificity of 83 per cent for detection of advanced SOS. The related negative predictive value was 91 per cent. In a subgroup of patients, the fractional extraction (FE) of HA by the liver could be calculated. The FE by the liver represents the percentage of HA that is actually taken up from the blood by the liver in relation to its inflow. Although there seemed to be a trend towards net hepatic uptake of HA in patients with no or mild SOS versus net hepatic release in patients with moderate or severe SOS, the FE of HA was not significantly different between these groups. Whether this was due to the large variation in fractional extraction of HA by the liver or actual preserved SEC function remains to be determined in a future study. Based on our results, we conclude that the increased systemic HA levels in patients with SOS did not result from decreased hepatic clearance or increased hepatic production, and may be explained by portosystemic shunting due portal hypertension or enhanced production at other sites than the liver. High HA levels identify patients at risk of SOS in whom additional investigations to detect the presence of SOS are indicted. Whether these patients benefit from restrictive liver surgery or two-stage hepatectomy combined with portal vein occlusion needs to be determined in future studies.

A recent publication showed that the presence of sinusoidal injury after oxaliplatin-based chemotherapy for CLMs was related to decreased recurrence-free and overall survival[50]. In patients with CLMs, disease-free and overall survival are correlated with the histological tumour response, reflected by the tumour regression grade (TRG)[51]. The TRG is a read out of the presence of residual tumour cells and fibrosis[51, 52]. We hypothesized that the decreased disease-free survival in patients with SOS would be related to a decreased tumour response in these patients. Indeed, in **chapter 9**, we demonstrated that the tumour response was significantly decreased in patients with SOS. We postulated that a mechanism contributing to the decreased tumour response in patients with SOS was hypoperfusion due to the obliterative lesions inducing oxidative stress, which is known to induce tumour cell invasion[53]. Moreover, the vascular damage may have led to decreased penetration of oxaliplatin to the tumour sites. Unfortunately, long-term survival data of the patients in our cohort were lacking, as they were operated in 2008 and 2009 only, and therefore, we could not confirm a relation between SOS, TRG and long-term survival to date.

Oxidative stress and endothelial injury play an important role in the development of SOS and may therefore be potential therapeutic targets[39]. In **chapter 10**, the protective effect of monoHER on the development of SOS was studied in an experimental rat model[54]. MonoHER is a flavonoid with favorable effects on the microvascular endothelium and anti-oxidative and anti-inflammatory properties, making it a promising drug for the prevention of SOS[55, 56]. MonoHER has proven to be effective in preventing doxorubicin-

induced cardiotoxicity, which is believed to be caused by the formation of oxygen free radicals[55]. In a validated rat model of SOS, pre-treatment with monoHER was able to prevent the development of portal hypertension, hepatocellular injury and endothelial cell damage after 72hr. Mechanisms underlying the protective effect of monoHER were related to interference with SEC injury, matrix metalloproteinase expression and influx of inflammatory cells. Of important notice, there was no effect of monoHER on the cytotoxic capacity of oxaliplatin *in vitro* in different colorectal cancer cell lines. Further studies are needed to clarify the effect of monoHER on the preservation of remnant liver function after 70 per cent partial hepatectomy in the experimental rat model. Moreover, assessment of the efficacy of monoHER on the prevention of oxaliplatin-induced SOS in man is warranted.

In summary, we illustrated in part III of this thesis that sinusoidal injury secondary to oxaliplatin for CLMs may lead to adverse short-term clinical outcomes. However, not only short-term, but also long-term outcomes may be affected, as the tumour response was significantly decreased in patients with moderate or severe SOS. Next, we showed that systemic HA levels had a fair diagnostic performance for the detection of SOS after oxaliplatin-based chemotherapy. Patients with high HA levels should undergo additional investigations into the presence of SOS. Finally, we demonstrated that monoHER was able to prevent the clinical consequences of SOS in an experimental rat model. It is of importance to translate this observation to man in order to improve post-resectional outcomes of patients with CLMs treated with oxaliplatin-based neoadjuvant chemotherapy.

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Chapter 12

Summary and discussion (in Dutch)

Een leveroperatie is de behandeling van keuze voor een groot aantal tumoren van de lever. De veiligheid van een leveroperatie is het afgelopen decennium toegenomen door vooruitgang op het gebied van patiëntenselectie, chirurgische techniek en postoperatieve zorg[1-3]. Door deze toegenomen veiligheid komen steeds meer patiënten met levertumoren in aanmerking voor een operatie.

Bij een leveroperatie wordt het deel van de lever dat tumor bevat, verwijderd. Zowel het volume als de functie van het overgebleven deel van de lever (de restlever) zijn na zo'n operatie tijdelijk afgenomen. Wanneer zij niet tijdig herstellen, kan postoperatief leverfalen (PLF) optreden. PLF wordt gekenmerkt door een verstoring van de fysiologische functies van de lever. Dit kan leiden tot een ophoping van giftige stoffen in het bloed en een verminderde aanmaak van essentiële eiwitten. In de inleiding van dit proefschrift (**hoofdstuk 1**) worden verschillende aspecten van PLF beschreven. Kort samengevat, ontwikkelt ongeveer één op de dertig patiënten symptomen van leverfalen na een leveroperatie. Risicofactoren voor het ontwikkelen van PLF zijn operatie- en patiëntgebonden. Leverfalen is de nummer één doodsoorzaak na een leveroperatie. Omdat er tot op heden geen goede behandelopties zijn, is het letterlijk van levensbelang om PLF te voorkomen.

Dit proefschrift heeft als doel de uitkomsten van leverchirurgie te analyseren en, waar mogelijk, te verbeteren. Specifieke aandacht wordt hierbij gegeven aan de risicofactoren voor het ontwikkelen van PLF. Dit vertaalt zich in de volgende drie doelen:
Doel 1. Ontwikkelen van methoden die de haalbaarheid vergroten van het verrichten van kwalitatief hoogwaardig onderzoek naar klinische uitkomsten van leverchirurgie (hoofdstuk 2, 3 en 4).
Doel 2. Evalueren van operatiegebonden risicofactoren voor PLF middels het bestuderen van het effect van twee verschillende chirurgische technieken op het ontwikkelen van levercel schade (hoofdstuk 5 en 6).
Doel 3. In kaart brengen van patiëntgebonden risicofactoren voor PLF, in het bijzonder schade aan de haarvaten van de lever als gevolg van chemotherapie in patiënten met leveruitzaaiingen van darmkanker (hoofdstuk 7, 8, 9 en 10).

DEEL I. ANALYSE VAN UITKOMSTEN VAN LEVERCHIRURGIE: KRITISCHE BESCHOUWING VAN HUIDIGE KLINISCHE EINDPUNTEN

Het eerste doel van dit proefschrift is het ontwikkelen van strategieën die de haalbaarheid vergroten van het verrichten van gerandomiseerd onderzoek naar klinische uitkomsten van leverchirurgie.

Geneeskunde op basis van wetenschappelijk bewijs, ook wel *evidence-based medicine* genoemd, is de gouden standaard in de hedendaagse patiëntenzorg. Richtlijnen voor

de zorg van chirurgische patiënten zijn bij voorkeur gebaseerd op resultaten van kwalitatief hoogwaardig onderzoek, zoals gerandomiseerde studies en meta-analyses. Op het gebied van de leverchirurgie is het afgelopen decennium slechts een beperkt aantal kwalitatief hoogwaardige studies uitgevoerd[4]. Een verklaring voor dit kleine aantal studies zou kunnen zijn dat het uitvoeren van gerandomiseerd onderzoek binnen de leverchirurgie uitdagend is[5]. Deze uitdaging is zowel praktisch als technisch van aard, zoals het verzamelen van voldoende proefpersonen binnen een acceptabele termijn en de selectie van een eindpunt dat de onderzoeksvraag betrouwbaar kan beantwoorden[6]. Traditioneel worden binnen de chirurgie klinische eindpunten, zoals overlijden (mortaliteit) of postoperatieve complicaties (morbiditeit), gekozen om het effect van een interventie te beoordelen[7]. Daarom focust deel I van dit proefschrift zich op het vergroten van de haalbaarheid van gerandomiseerd onderzoek dat gebruik maakt van klinische eindpunten van leverchirurgie.

In **hoofdstuk 2** werd de haalbaarheid van het verrichten van een gerandomiseerde studie binnen de leverchirurgie met een klinisch eindpunt als primair eindpunt geëvalueerd. Op basis van literatuuronderzoek werd het gemiddelde voorkomen (incidentie) van mortaliteit en morbiditeit na een leveroperatie berekend in de periode tussen 2002 en 2007. De gemiddelde mortaliteit was 1.0 procent en de gemiddelde morbiditeit 28.9 procent; PLF had een incidentie van 2.6 procent. De groepsgrootte voor een gerandomiseerde studie werd berekend op basis van de gevonden incidentiegetallen. In het algemeen geldt dat de groepsgrootte die nodig is voor een studie groter is, naarmate de incidentie van het eindpunt dat bestudeerd wordt, lager is[8]. We berekenden dat er ongeveer 12 000 patiënten nodig zouden zijn voor een studie die tot doel had een één derde vermindering van de incidentie van PLF aan te tonen. Omdat in alle Nederlandse ziekenhuizen samen jaarlijks slechts 500 leveroperaties worden uitgevoerd, concludeerden wij dat de haalbaarheid van het verrichten van een gerandomiseerde studie met een klinisch eindpunt zoals PLF minimaal is. Wij stellen voor om samengestelde eindpunten te gebruiken, multicentrische studies op te zetten of meta-analyses uit te voeren om de haalbaarheid van kwalitatief hoogwaardig onderzoek dat gebruik maakt van klinische eindpunten na leverchirurgie te vergroten. Als alternatief zouden surrogaat eindpunten gebruikt kunnen worden, echter robuuste validatie van deze eindpunten binnen de leverchirurgie ontbreekt tot op heden[9].

Voor het gebruik van de eerder genoemde samengestelde eindpunten of het verrichten van meta-analyses zijn uniforme definities van klinische eindpunten noodzakelijk[10]. Dit is belangrijk omdat anders het risico bestaat dat twee totaal verschillende condities met elkaar vergeleken worden. In **hoofdstuk 3** werden uniforme definities van klinische eindpunten na leverchirurgie geformuleerd. Middels een tweede literatuuronderzoek ontdekten we dat duidelijke definities van klinische uitkomsten, die gebruikt werden als eindpunten van gerandomiseerde studies in de leverchirurgie, in minder dan één derde

van de artikelen werden beschreven. Als zij beschreven werden, bleken de definities van eenzelfde klinische uitkomstmaat substantieel te verschillen tussen artikelen. De definities die we tijdens het literatuuronderzoek vonden, vormden de basis van een elektronische enquête. Deze enquête werd verspreid onder 54 internationaal vooraanstaande leverchirurgen in een poging consensus te bereiken over de definitie van de meest gebruikte klinische eindpunten. De enquête werd door 31 chirurgen ingevuld (respons percentage van 57 procent) en leidde tot aanpassingen van de voorgestelde definities op basis van het commentaar van de respondenten. Zo ontstonden er gestandaardiseerde definities van de meest frequent gebruikte klinische eindpunten van leverchirurgie. Voordat deze definities wereldwijd geïmplementeerd kunnen worden, moeten zij door een nog grotere groep van experts worden beoordeeld en in een grote patiëntengroep worden getest (validatie).

Vooruitlopend op deze validatie, werden de geformuleerde definities alvast gebruikt in **hoofdstuk 4** om een leverchirurgie specifiek samengesteld eindpunt te ontwikkelen. Een samengesteld eindpunt is een combinatie van twee of meer klinische uitkomstmaten, die als één eindpunt worden beschouwd[11]. Samengestelde eindpunten leiden tot een groter onderscheidingsvermogen (*power*) en efficiëntie, omdat zij een hogere incidentie hebben dan een enkelvoudig klinisch eindpunt. Dientengevolge zal de groepsgrootte van een studie die een samengesteld eindpunt als primair eindpunt gebruikt, kleiner zijn. Op basis van voorgenoemde elektronische enquête werd een goed gedefinieerd en zorgvuldig gecomponeerd leverchirurgie specifiek samengestelde eindpunt ontwikkeld, bestaand uit de individuele componenten buikwaterzucht (ascites), PLF, gallekkage, intra-abdominale bloeding, intra-abdominaal abces en mortaliteit. Eén van de voorwaarden voor de betrouwbaarheid van een samengesteld eindpunt is dat de incidentie van de componenten vergelijkbaar is[12, 13]. Daarom werd de incidentie van de individuele componenten van het leverchirurgie specifiek samengestelde eindpunt geëvalueerd in 815 patiënten die een leveroperatie ondergingen in twee Europese levercentra. Het voorkomen van de individuele componenten varieerde bij benadering tussen de 1 en 10 procent. Het samengestelde eindpunt had een incidentie van 11 procent in het ene en 19 procent in het andere cohort. De verdubbeling van de incidentie van het leverchirurgie specifiek samengestelde eindpunt ten opzicht van de individuele componenten, leidde grofweg tot een halvering van de benodigde patiëntenaantallen in een gerandomiseerde studie. De bruikbaarheid van het leverchirurgie specifiek samengestelde eindpunt is dus veelbelovend, echter ook hier geldt dat aanvullende evaluatie gewenst is.

Samenvattend tonen de resultaten van deel I van dit proefschrift dat de haalbaarheid van gerandomiseerd onderzoek binnen de hedendaagse leverchirurgie, dat gebruik maakt van klinische eindpunten, laag is als gevolg van de grote patiëntenaantallen die nodig zijn voor voldoende *power.* De oplossing kan gezocht worden in het uitvoeren

van meta-analyses of de implementatie van een goed gedefinieerd leverchirurgie specifiek samengesteld eindpunt dat bestaat uit ascites, PLF, gallekkage, intra-abdominale bloeding, intra-abdominaal abces en operatieve mortaliteit. Aanvullende evaluatie van dit eindpunt is gewenst.

DEEL II. ANALYSE VAN UITKOMSTEN VAN LEVERCHIRURGIE: LEVERCEL SCHADE ALS GEVOLG VAN CHIRURGISCHE TECHNIEK

Het evalueren van het effect van verschillende chirurgische technieken op levercel schade in patiënten die een leveroperatie ondergaan, is het tweede doel van dit proefschrift.

Grote hoeveelheden bloedverlies tijdens een leveroperatie zijn geassocieerd met nadelige korte en lange termijn uitkomsten[14-16]. Zowel het mobiliseren van de lever als het afwisselend afklemmen en openzetten van de bloedtoevoer naar de lever (intermitterende Pringle manoeuvre) worden toegepast om het bloedverlies tijdens een leveroperatie te beperken. Het gebruik van deze technieken staat ter discussie, omdat ze beide zelf ook weer aanzienlijke levercel schade veroorzaken, hetgeen tot PLF zou kunnen leiden[17-19]. Om deze levercel schade beter te karakteriseren, worden in deel II van dit proefschrift beide technieken afzonderlijk bestudeerd in patiënten die een leveroperatie ondergaan.

Allereerst het mobiliseren van de lever: dit omvat het losmaken van de lever van de omgevende structuren zoals de buikwand, het middenrif en de onderste holle ader. Het doel van het mobiliseren van de lever tijdens een leveroperatie is het bloedverlies uit aders die vanuit de lever naar deze omgevende structuren lopen, te controleren. Onze onderzoeksgroep toonde eerder al aan dat mobilisatie van de lever de belangrijkste oorzaak van levercel schade tijdens een leveroperatie was. Tevens lieten we al zien dat het mobiliseren van de lever gevolgd werd door een ontstekingsreactie in het gehele lichaam[20]. Proefdiermodellen suggereren dat stimulatie van het immuunsysteem in de lever een belangrijke rol speelt bij het ontwikkelen van mobilisatie-geassocieerde levercel schade[21]. Echter, in de mens is deze relatie nog nooit aangetoond. Het is belangrijk om meer inzicht te krijgen in rol van het immuunsysteem bij het ontstaan van levercel schade door mobilisatie, omdat de ontstekingsreactie beïnvloed kan worden door bijvoorbeeld ontstekingsremmende medicatie. Daarom werd in **hoofdstuk 5** de link tussen mobilisatie van de lever, levercel schade en ontsteking in de lever onderzocht bij 25 patiënten die een leveroperatie ondergingen. Wij toonden aan dat er een verband was tussen mobilisatie van de lever en ontsteking in de lever. Deze ontsteking werd gekenmerkt door de aanwezigheid van een groot aantal ontstekingscellen in de lever en een verhoogde expressie van genen die betrokken zijn bij de productie van ontstekingseiwitten. Tevens vonden wij een significante relatie tussen de duur van de mobilisatie en

het aantal ontstekingscellen dat zich in de lever ophoopte. De groepsgrootte van deze studie was te klein om een relatie tussen mobilisatie-geïnduceerde levercel schade en klinische eindpunten van leverchirurgie vast te stellen. Andere onderzoekers lieten echter al zien dat het voorkomen van PLF de neiging had lager te zijn in patiënten die een leveroperatie ondergingen via de zogenaamde anterieure benadering[22]. De anterieure benadering wordt ook wel de *no touch* techniek genoemd, omdat bij deze techniek de lever niet geheel wordt losgemaakt uit zijn omgeving. Op basis van voorgenoemde data veronderstellen wij dat het klinisch relevant zou kunnen zijn om levercel schade door mobilisatie te voorkomen. Preventie zou gelegen kunnen zijn in het toepassen van de eerdergenoemde anterieure benadering of het toedienen van ontstekingsremmende medicatie tijdens een leveroperatie[18, 23-25].

Na het mobiliseren van de lever volgt in de regel het doorsnijden van het leverweefsel om de levertumor te verwijderen. Een van de manieren om het bloedverlies tijdens het doornemen van het leverweefsel te verminderen, is de intermitterende Pringle manoeuvre[26]. De intermitterende Pringle manoeuvre behelst het afwisselend afklemmen en openzetten van de bloedtoevoer naar de lever. De bloedtoevoer naar de lever wordt verzorgd door twee aders: de leverslagader, die zuurstofrijk bloed direct uit de grote lichaamsslagader naar de lever vervoert, en de poortader, die zuurstofarm bloed uit de darm naar de lever vervoert. Tijdens de intermitterende Pringle manoeuvre worden beide aders afgesloten en wordt de lever een periode onvoldoende van bloed voorzien (ischemie), waarna de bloedtoevoer weer wordt hersteld (reperfusie). De optimale duur van de ischemische intervallen tijdens de Pringle manoeuvre staat nog ter discussie. Gewoonlijk wordt het effect van verschillende ischemische intervallen tijdens de Pringle manoeuvre bestudeerd op basis van het gehalte leverenzymen in het bloed op verschillende dagen na een leveroperatie. Het is echter maar zeer de vraag of deze markers wel gevoelig genoeg zijn om klinisch relevante verschillen in levercel schade als het gevolg van de intermitterende Pringle manoeuvre te detecteren[27]. Het intra-operatief monitoren van de plasma concentratie van het meer specifieke eiwit *liver fatty acid-binding protein* (L-FABP) is mogelijk een meer accurate manier om verschillen in levercel schade te bepalen. L-FAPB is een klein eiwit met een lage molecuul massa dat in de cel betrokken is bij het transport en metabolisme van vetten[28, 29]. Na leverschade stijgt het L-FABP gehalte in het bloed vlug omdat L-FAPB snel wordt vrijgemaakt uit beschadigde levercellen[30]. In **hoofdstuk 6** toonden we in een gerandomiseerde studie bij de mens aan dat een Pringle manoeuvre met 30 minuten durende ischemische intervallen leidde tot vergelijkbare levercel schade, gebaseerd op het L-FABP gehalte in het bloed, als een Pringle manoeuvre met 15 minuten durende ischemische intervallen. Daarbij was ook de postoperatieve leverfunctie gelijkwaardig tussen de twee groepen. Bovendien deden we de belangrijke bevinding dat het niet toepassen van de Pringle manoeuvre leidde tot significant minder levercel schade. Er wordt vaak gesuggereerd dat de Pringle

manoeuvre kan leiden tot darmschade als gevolg van het afklemmen van de poortader, echter hiervoor vonden wij in deze studie geen bewijs. In een follow-up studie van onze onderzoeksgroep vonden we echter wel aanwijzingen voor een verhoogde aanwezigheid van bacteriële producten in het bloed na een Pringle manoeuvre, hetgeen zou kunnen wijzen op een verstoring van de darm barrière[31]. Omdat in een recente meta-analyse geen klinisch voordeel werd gevonden van het gebruik van de Pringle manoeuvre[17], adviseren wij in principe geen Pringle manoeuvre te gebruiken tijdens het doorsnijden van het leverweefsel. Wanneer het noodzakelijk is om een Pringle manoeuvre te gebruiken, kunnen 30 minuten durende ischemische intervallen worden gekozen.

Concluderend toont deel II van dit proefschrift dat mobilisatie van de lever geassocieerd is met levercel schade en ontsteking in de lever, proportioneel aan de duur van de mobilisatie. Of het verminderen van de levercel schade of de ontstekingsreactie bij de mens gunstige effecten heeft, moet onderzocht worden in toekomstige studies. Daarnaast laten we zien dat een Pringle manoeuvre met 15 of 30 minuten durende ischemische intervallen leidt tot vergelijkbare levercel schade en postoperatieve leverfunctie. Echter, het niet toepassen van de Pringle manoeuvre leidt tot significant minder schade en daarom zou de Pringle manoeuvre terughoudend gebruikt moeten worden tijdens leverchirurgie.

DEEL III. ANALYSE VAN UITKOMSTEN VAN LEVERCHIRURGIE: LEVERCEL SCHADE ALS GEVOLG VAN CHEMOTHERAPIE

Het derde doel van dit proefschrift is het bestuderen van vaatschade aan de lever als gevolg van chemotherapie in patiënten met leveruitzaaiingen van darmkanker.

Voor patiënten met leveruitzaaiingen van darmkanker, ook wel colorectale levermetastasen (CLMs) genoemd, is een operatie aan de lever, waarbij alle uitzaaiingen worden verwijderd, de enige behandeling met kans op genezing[32]. Helaas komt slechts de minderheid van patiënten met CLMs in eerste instantie in aanmerking voor een operatie, omdat de uitzaaiingen zich in de gehele lever of zelfs daarbuiten bevinden. Daarom is een multidisciplinaire behandeling bestaande uit chemotherapie met capecitabine, oxaliplatin of irinotecan gevolgd door een leveroperatie, in deze groep tegenwoordig meer regel dan uitzondering. Bij 15 tot 30 procent van de patiënten met uitgebreide leveruitzaaiingen leidt behandeling met chemotherapie tot een verkleining van de tumormassa, waardoor een leveroperatie alsnog mogelijk wordt[33]. Het veelgebruikte chemotherapeuticum oxaliplatin kent echter serieuze bijwerkingen. Een van deze bijwerkingen is schade aan de haarvaten (sinusoïden) van de lever. Deze schade staat bekend als het sinusoïdaal obstructie syndroom (SOS)[34-36]. De aanwezigheid van SOS is geassocieerd met een verhoogde kans op complicaties na een uitgebreide leveropera-

tie, zoals het ontwikkelen van en overlijden aan PLF[36-38]. In deel III van dit proefschrift bestuderen we daarom de effecten van oxaliplatin op de haarvaten van de lever.

In **hoofdstuk 7** lieten we aan de hand van twee casussen zien waartoe de aanwezigheid van sinusoïdale schade als gevolg van oxaliplatin kan leiden in patiënten die een uitgebreide leveroperatie ondergingen. Beide patiënten hadden geen onderliggende leverziekte en werden in verband met leveruitzaaiingen van darmkanker behandeld met oxaliplatin voor de leveroperatie. Analyse van het leverweefsel na de operatie toonde uitgebreide sinusoïdale schade. Door deze schade was de doorstroming van het bloed in de lever verstoord, hetgeen leidde tot een verhoogde druk in de poortader (portale hypertensie)[39]. Portale hypertensie is een zorgwekkend ziektebeeld, omdat dit tot levensbedreigende complicaties zoals maag- en slokdarmbloedingen kan leiden. Deze complicaties traden bij beide patiënten op en leidden tot het overlijden van één van hen. Hoewel in dit hoofdstuk het klinische beloop van slechts twee patiënten wordt beschreven, is het probleem van sinusoïdale schade na oxaliplatin groot. SOS ontstaat in ongeveer twee tot zeven op de tien patiënten die met oxaliplatin worden behandeld wegens leveruitzaaiingen[35, 36, 38, 40]. Gezien de schade die de behandeling met oxaliplatin met zich meebrengt, is het belangrijk het voordeel van het gebruik van oxaliplatin per patiënt af te zetten tegen het risico op het ontwikkelen van sinusoïdale schade. Op basis van deze afweging zou bepaald moeten worden of het gebruik van oxaliplatin voor een leveroperatie daadwerkelijk noodzakelijk is. Als dit het geval is, zoals bij patiënten die in eerste instantie niet in aanmerking komen voor een leveroperatie, is het van belang het ontstaan van deze schade vroegtijdig te herkennen en zo mogelijk te voorkomen.

Vroegtijdige herkenning van sinusoïdale schade als gevolg van oxaliplatin is belangrijk, omdat het operatieplan hierdoor kan veranderen. Patiënten met SOS ondergaan bij voorkeur een minder uitgebreide operatie of een operatie in twee stappen, omdat de functie van de restlever is aangetast. De huidige detectiemethoden voor SOS maken gebruik van indirecte markers voor vaatschade en hebben daarom een bescheiden sensitiviteit en specificiteit[36, 38, 40]. In **hoofdstuk 8** werd bestudeerd of het meten van het hyaluronzuur (HZ) gehalte in het bloed een meer betrouwbare maat zou kunnen zijn voor het ontdekken van SOS in patiënten die behandeld zijn met oxaliplatin. HZ wordt in onze gewrichten geproduceerd. Na afgifte van HZ aan de bloedbaan wordt het vrijwel volledig opgenomen en verwerkt door de sinusoïdale cellen (SCs) in de haarvaten van de lever[41, 42]. Derhalve wordt het HZ gehalte in het bloed gezien als maat voor de functie van deze SCs. Juist deze SCs zijn beschadigd in patiënten met SOS en dus veronderstelden wij dat het HZ gehalte in het bloed verhoogd zou zijn in deze groep. In patiënten die tussen 2008 en 2009 in het Maastricht Universitair Medisch Centrum een leveroperatie ondergingen na behandeling met oxaliplatin, toonde het leverweefsel in 58 procent tekenen van SOS. Het HZ gehalte van het bloed was significant verhoogd in patiënten met SOS in vergelijking met patiënten zonder SOS. Bij een grenswaarde van

44.1 ng/mL had HZ een sensitiviteit van 67 procent en een specificiteit van 83 procent voor de detectie van SOS. De negatief voorspellende waarde was 91 procent. Op basis van deze getallen concludeerden we dat de kans op sinusoïdale schade heel klein is, als het HZ gehalte onder de grenswaarde van 44.1 ng/mL ligt. Dit houdt concreet in dat aanvullend onderzoek naar de aanwezigheid van SOS niet noodzakelijk is in deze patiënten. Aanvullend onderzoek is wel aangewezen in patiënten met een HZ gehalte boven de grenswaarde van 44.1 ng/mL. Dit aanvullend onderzoek zou bijvoorbeeld kunnen bestaan uit het verkrijgen van leverweefsel via een biopsie. Of patiënten met een verhoogde HZ concentratie uiteindelijk baat hebben bij een beperktere leveroperatie, moet in de toekomst blijken. Om een verklaring te vinden voor het verhoogde HZ gehalte in het bloed bij patiënten met SOS, voerden we een deelstudie uit. Een verhoogde HZ waarde moet namelijk het gevolg zijn van ofwel een verhoogde aanmaak van HZ ofwel een verminderde afbraak van HZ. Verrassend genoeg vonden we geen bewijs voor een verminderde afbraak van HZ door de beschadigde SCs in de lever van patiënten met SOS.

Een recente studie laat zien dat de lange termijn overleving van patiënten met SOS verminderd is ten opzichte van patiënten zonder SOS na oxaliplatin-bevattende chemotherapie wegens leveruitzaaiingen van darmkanker[43]. De lange termijn overleving van patiënten met leveruitzaaiingen wordt onder andere bepaald door de reactie van deze uitzaaiingen op chemotherapie[44]. Deze reactie wordt weergegeven als de tumor regressie graad (TRG)[45]. De TRG wordt bepaald door het aantal vitale tumorcellen in leveruitzaaiingen onder de microscoop te bestuderen. Wij veronderstelden dat de tumor respons in patiënten met SOS afgenomen zou zijn, hetgeen de verminderde lange termijn overleving zou kunnen verklaren. In **hoofdstuk 9** leverden we inderdaad het bewijs dat de aanwezigheid van SOS leidde tot een verminderde tumor respons op oxaliplatin. Een verklaring voor de afname van het celdodende effect van oxaliplatin in patiënten met SOS zou kunnen zijn dat de vaatschade in de lever leidt tot een ongunstig tumor milieu en een verminderde concentratie van het chemotherapeuticum op de plaats van de leveruitzaaiing. Helaas konden we in deze studie (nog) geen relatie met de lange termijn overleving leggen omdat onze patiënten tussen 2008 en 2009 werden geopereerd. Wanneer de 5-jaars follow-up is bereikt, is analyse van het effect van SOS en tumor regressie graad op de ziektevrije en totale overleving een belangrijke volgende stap om onze hypothese te bevestigen.

In de ontwikkeling van SOS spelen oxidatieve stress, ontsteking en vaatschade een belangrijke rol[34]. Deze processen zijn dan ook belangrijke therapeutische aanknopingspunten. In **hoofdstuk 10** bestudeerden we het beschermende effect van het flavonoïde monoHER op het ontstaan van SOS in een proefdiermodel[46]. MonoHER bezit verschillende gunstige kenmerken, zoals een beschermend effect op de vaatwand en ontstekingsremmende en antioxidatieve eigenschappen[47, 48]. Recent is reeds aangetoond dat

monoHER beschermend werkt tegen chemotherapie-geïnduceerde hartcel schade, welke ook wordt veroorzaakt door oxidatieve stress[47]. In ons proefdiermodel was het flavonoïde monoHER in staat om de ontwikkeling van vaatschade, levercel schade en portale hypertensie te voorkomen. Tevens toonden we in celkweken aan dat monoHER geen effect had op de celdodende capaciteit van oxaliplatin. Aanvullende studies zijn nodig om de effectiviteit van monoHER te bestuderen in een proefdiermodel waarin een leveroperatie wordt uitgevoerd. Wanneer monoHER ook dan effectief blijkt, is een studie in de mens een logische volgende stap.

Samenvattend laten we in deel III van dit proefschrift zien dat vaatschade in de lever als gevolg van oxaliplatin kan leiden tot ongunstige korte termijn uitkomsten. Ook leveren we bewijs dat de respons van leveruitzaaiingen op oxaliplatin verminderd is in patiënten met SOS. Dit zou invloed kunnen hebben op de lange termijn uitkomsten van patiënten met CLMs en SOS, echter dit moet onderzocht worden in toekomstige studies. Het HZ gehalte in het bloed kan worden gebruikt om patiënten met een verhoogd risico op SOS preoperatief te identificeren. Of deze patiënten profijt hebben van een beperkte leveroperatie moet in de toekomst blijken. Tot slot laten we zien dat het flavonoïde monoHER in staat is om vaatschade en levercel schade in een proefdiermodel te voorkomen. Het is van groot belang deze observatie te vertalen naar de mens om de uitkomsten van leverchirurgie na oxaliplatin-bevattende chemotherapie te verbeteren.

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Chapter 13

Clinical implications

PART I. ASSESSING OUTCOMES OF LIVER SURGERY: CRITICAL APPRAISAL OF CLINICAL ENDPOINTS

Observation:

In current HPB practice, liver surgery-related mortality and morbidity rates are low: operative mortality has a mean rate of 1 per cent and the mean rates of specific types of morbidity are all below 8 per cent. Consequently, the sample size required for an adequately powered randomized controlled trial (RCT) in liver surgery using surgery-related mortality or morbidity as primary endpoint turns out to be extremely large.

Implication:

The feasibility of RCTs in liver surgery using clinical outcomes as primary endpoint is low as such endpoints require large sample sizes owing to their low incidence. Strategies that may enable the conduct of high level of evidence research in liver surgery using clinical outcomes include the use of composite endpoints, organization of multicentre trials and performance of meta-analyses.

Observation:

Clear definitions of clinical outcomes, used as primary or secondary endpoints in RCTs in liver surgery, are provided in less than one-third of trial reports. If provided, the definitions differ substantially between studies.

Implication:

The lack of uniform definitions hampers the interpretation of trial results and compromises the validity of systematic reviews and meta-analyses in HPB surgery. Our attempt to design consensus-based standard definitions of clinical outcomes of liver surgery may solve this problem. Prospective validation of the proposed definitions is warranted.

Observation:

The use of a composite endpoint (CEP) as primary endpoint of RCTs in liver surgery is not very common at present and uniformity of CEP components is lacking. A well-defined liver surgery specific CEP, consisting of ascites, post-resectional liver failure, bile leakage, intra-abdominal abscess, intra-abdominal hemorrhage and operative mortality is proposed. This liver surgery specific CEP has a considerably higher event rate than any of its individual components.

Implication:

Implementation of a liver surgery specific CEP potentially increases the feasibility of conducting RCTs using clinical outcomes of liver surgery as primary endpoint. Its significantly higher incidence rate will decrease the number of patients needed for an adequately powered trial. It is important to keep in mind that the use of CEPs can be misleading if components vary widely in clinical relevance or incidence. Therefore, components should be classified using an objective severity grading system and the incidence of all components should be reported. Prospective validation of the proposed liver surgery specific CEP is warranted.

PART II. ASSESSING OUTCOMES OF LIVER SURGERY: HEPATIC DAMAGE AS A RESULT OF SURGICAL TECHNIQUE

Observation:

Liver mobilization during liver surgery in man is associated with hepatocellular damage and liver inflammation. The amount of liver inflammation is significantly associated with the duration of inflammation.

Implication:

As mobilization-induced hepatocellular damage might have a negative impact on short-term outcomes of liver surgery, preventive strategies may be beneficial. They include either employment of alternative surgical techniques, such as the anterior approach or laparoscopic surgery, or modulation of inflammation by anti-inflammatory drugs.

Observation:

An intermittent Pringle maneuver using 30 min ischemic intervals induces a similar amount of hepatocellular damage, reflected by systemic liver fatty acid-binding protein levels, compared to 15 min ischemic intervals, without loss of remnant liver function. No Pringle maneuver leads to significantly less liver cell damage.

Implication:

It is preferable not to use inflow occlusion during liver resection. If inflow occlusion is required, 30 minutes ischemic intervals may be used.

PART III. ASSESSING OUTCOMES OF LIVER SURGERY: HEPATIC DAMAGE AS A RESULT OF OXALIPLATIN-BASED CHEMOTHERAPY

Observation:

Oxaliplatin-based neoadjuvant chemotherapy causes sinusoidal injury of the non-tumour-bearing liver consistent with nodular regenerative hyperplasia. This condition is associated with the development of portal hypertension.

Implication:

In patients with colorectal liver metastases (CLMs), the indication for neoadjuvant chemotherapy treatment should be evaluated carefully by a multidisciplinary team. The benefit of tumour downsizing should be weighed against the disadvantage of induction of sinusoidal injury. If deemed necessary, caution should be exercised in patients treated with oxaliplatin-based chemotherapy prior to major liver surgery. Thorough analysis of liver volume and function should guide the operative strategy. When future remnant liver volume is regarded insufficient, portal vein embolization, two stage hepatectomy, ALPPS approach or restrictive surgery should be considered.

Observation:

Elevated systemic hyaluronic acid levels have a fair diagnostic performance for the non-invasive detection of patients with sinusoidal obstruction syndrome (SOS) after oxaliplatin-based chemotherapy for CLMs. The net functional clearance capacity of hepatic sinusoidal endothelial cells (SECs) for hyaluronic acid seems to be preserved in patients with SOS.

Implication:

High systemic hyaluronic acid levels identify patients at risk of SOS. In these patients, additional investigations into the presence of SOS are indicated. The presence of sinusoidal injury *per se* does not seem to interfere with the capacity of hepatic SECs to clear hyaluronic acid. From a mechanistic point of view, it would be relevant to obtain a more detailed insight into hyaluronic acid metabolism and SEC function in patients with SOS secondary to oxaliplatin-based chemotherapy by either performing hyaluronic acid loading tests or PET scans with [^{11}C]hyaluronic acid.

Observation:

The incidence of SOS of the non-tumour-bearing liver in patients with CLMs treated with oxaliplatin-based chemotherapy prior to surgery is approximately 60 per cent. The tumour regression grade is increased in patients suffering from SOS.

Implication:

An increased tumour regression grade is consistent with a decreased tumour response to chemotherapy. Tumour regression grade and tumour response are important for the prediction of long-term survival and consequently, patients with SOS who exhibit a decreased tumour response may have an unfavorable long-term clinical outcome. Therefore, it seems to be clinically relevant to develop strategies that prevent SOS secondary to oxaliplatin-based chemotherapy.

Observation:

MonoHER, a flavonoid with antioxidant, anti-inflammatory and vaso-active properties, is able to prevent the development of portal hypertension, hepatocellular damage and hepatic sinusoidal endothelial cell injury in an experimental rat model of SOS. Moreover, there is no effect of monoHER on the cytotoxic capacity of oxaliplatin *in vitro* in different colorectal cancer cell lines.

Implication:

Administration of monoHER is successful in preventing SOS in an experimental rat model. Phase I and II clinical trials with monoHER have been performed in man without evidence for serious side effects of monoHER administration. Translation of our experimental observations to man is warranted as validated strategies to prevent sinusoidal injury secondary to oxaliplatin-based chemotherapy for CLMs in man are lacking to date.

Chapter 14

Future perspectives

The broader aim of the present thesis was to assess and improve outcomes of liver surgery. The studies described in this thesis focused on the conduct of high level of evidence research in liver surgery as well as surgery and patient-related risk factors of post-resectional liver failure (PLF). Based on the results of the present thesis, several clinical questions have been answered. However, probably an even larger number of questions have been generated that warrant future investigation. Some interesting, unresolved issues with high clinical relevance will be discussed shortly in the next paragraphs.

ARE WE USING THE RIGHT OUTCOMES AS ENDPOINTS OF RANDOMIZED CONTROLLED TRIALS IN LIVER SURGERY?

The conduct of appropriately powered randomized controlled trials (RCTs) in liver surgery using clinical outcomes as primary endpoints is hardly feasible due to declining event rates (chapter 2). In order to overcome this problem, composite or surrogate endpoints may be used as primary endpoints. Whereas the pros and cons of the use of composite endpoints (CEPs) have been extensively discussed in the present thesis (chapter 4), the use of surrogate endpoints (SEPs) has not. The US Food and Drug Administration defined SEPs as "laboratory measurements or physical signs that are used in therapeutic trials as substitutes for true, clinically meaningful endpoints that are direct measures of how a patient feels, functions or survives, and are expected to predict the effect of therapy". The use of SEPs is attractive as they potentially reduce the required sample size, costs and inclusion periods of RCTs, which allows more rapid testing and implementation of treatment strategies[1-3].

SEPs that are frequently used in liver surgery-related trials include hepatocellular damage markers such as aminotransferases as surrogates for liver surgery-related morbidity, hepatic function markers such as bilirubin level or prothrombin index as surrogates for PLF, or progression-free survival as surrogate for overall survival (see [4-6]). Unfortunately, robust validation of these commonly used SEPs in liver surgery is lacking to date. In 1989, Prentice described four criteria for the identification of valid SEPs. These criteria can be summarized as follows: (1) the intervention has a statistically significant impact on the SEP, (2) the intervention has a statistically significant impact on the true, clinical endpoint, (3) the SEP has a statistically significant impact on the true, clinical endpoint, and (4) the full effect of the treatment on the true, clinical endpoint must be captured by the SEP[1, 3]. In several fields in medicine, critical appraisal of frequently used surrogates has been performed using these Prentice criteria[3, 7]. In liver surgery, such a formal validation of the commonly used SEPs is urgently warranted. This could be achieved by statistical analysis of combined data from multiple large RCTs in liver surgery that recorded both the occurrence of the surrogate and clinical endpoint. Up

until formal validation, the effect of the intervention on the true, clinical endpoint in liver surgery-related trials using a SEP as primary outcome parameter should be cautiously considered.

IS MODULATION OF INFLAMMATION BENEFICIAL IN PREVENTING MOBILIZATION-INDUCED HEPATOCELLULAR DAMAGE?

We showed that manipulation of the liver during liver surgery in humans was accompanied by hepatocellular damage, increased influx of inflammatory cells, upregulation of cellular adhesion molecules and enhanced expression of pro-inflammatory cytokines (chapter 5). Experimental studies have delivered convincing evidence that inflammation was causally involved in the process of mobilization-induced hepatocellular damage[8, 9]. When the inflammatory response was blunted by either depletion of Kupffer cells or modulation of Kupffer cell activity, liver cell damage decreased and clinical outcomes improved[9, 10]. From this, we speculate that modulation of inflammation by administration of agents that inhibit the activation or influx of cells that respond first to tissue injury, i.e. macrophages, neutrophils and mast cells, would also be beneficial in man[11]. Potential drugs that dampen inflammation include neutralizers of endogenous cytokines (e.g. anti-tumour necrosis factor-α), inhibitors of inflammatory cell influx (e.g. intracellular adhesion molecule antibodies), and stabilizers of mast cells or antagonists of mast cell releasate (e.g. antihistamines)[12, 13]. With respect to the latter, several lines of evidence in the field of manipulation-induced intestinal injury suggest that mast cells are involved in the attraction of inflammatory cells to the site of manipulation, whereas their depletion or inhibition of degranulation was effective in preventing intestinal inflammation in rodents and man[13, 14]. As mast cells are abundantly present in the liver[15], inhibition of their degranulation or blocking of certain histamine receptors during manipulation of the liver may be beneficial in preventing manipulation-induced hepatocellular damage and inflammation. We propose to study the role of mast cells and effect of mast cell stabilizers in an experimental model of liver manipulation. If proven effective, these results could be translated to patients undergoing surgery requiring manipulation of the liver in a randomized controlled fashion.

WHICH MEASURES ARE ABLE TO PREVENT SINUSOIDAL INJURY SECONDARY TO OXALIPLATIN-BASED CHEMOTHERAPY IN PATIENTS WITH COLORECTAL LIVER METASTASES?

Oxaliplatin-based chemotherapy is a pivotal element in the multimodal approach to patients with colorectal liver metastases (CLMs). One of the side-effects of oxaliplatin is injury to hepatic sinusoidal endothelial cells (SECs), characterized as sinusoidal obstruction syndrome (SOS). The presence of SOS is related to enhanced post-resectional morbidity after major liver surgery (chapter 7)[16, 17]. Endothelial cell damage, inflammation and oxidative stress all are involved in the pathophysiology of SOS[18]. Intravenous or intraperitoneal administration of anti-oxidants, vasoprotectors, nitric oxide-analogues, or matrix metalloproteinase inhibitors proved to be effective in preventing SOS in an experimental rat model[19-22]. We showed that intraperitoneal injection of monoHER was able to prevent SOS in rats (chapter 10). Moreover, we found no effect of monoHER on the cytotoxicity of oxaliplatin *in vitro* in colon cancer cell lines. However, evidence of the efficacy of aforementioned agents in preventing SOS in man is lacking to date.

To confirm our results on the effectiveness of monoHER in preventing SOS in man, we propose to conduct a clinical phase II study in patients with CLMs treated with oxaliplatin-based neoadjuvant chemotherapy. A clinical phase I study on the safety of the effective intravenous monoHER dose in healthy volunteers has already been performed[23]. The proposed phase II study should aim at (1) determination of the optimal dose and timing of monoHER administration, (2) assessment of the protective effect of monoHER on SOS by analysis of circulating endothelial damage markers and histological evidence of sinusoidal injury, (3) evaluation of the effect of monoHER on objective tumour response, and (4) analysis of the effect of monoHER on the preservation of remnant liver function[24].

In addition, other promising agents to protect the liver from sinusoidal injury secondary to oxaliplatin-based chemotherapy exist, such as intravenous administration of bevacizumab or oral aspirin treatment[25-27]. With respect to the latter, aspirin has been shown to prevent SOS after oxaliplatin-based chemotherapy in two independent, non-randomized studies in humans[27, 28]. The mechanism underlying the protective effect of aspirin is unknown and may include inhibition of platelet aggregation as well as modulation of inflammation[29]. As the administration of aspirin is simple, safe, and inexpensive, an RCT analyzing the effect of oral aspirin treatment on sinusoidal injury and post-resectional outcomes in patients with CLMs receiving oxaliplatin-based chemotherapy seems to be a next, logical step.

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Appendices

APPENDIX 1. CHECKLIST FOR THE SELECTION OF ARTICLES FOR FINAL ANALYSIS.

Details of article
Title
First author
Institute
Year of publication

Prerequisites for inclusion	**yes**	**no**
*More than 50 patients		
*More than 15 patients/year		
*All patient categories		
*All hepatectomies		
*Published between 2002-2007		
*No review or case report		
*No RCT with exclusion criteria		

RCT, randomized controlled trial; * item used for article selection.

Article review			**Quality score**
*Study design	RCT		5
	cohort study	prospective	3
		retrospective	1
*Inclusion period			1
*Number of patients			1
*Number of hepatectomies			
*Exclusion criteria			
*Mortality (%)			1
*Mortality index used	30-day	in-hospital	1
	operative	not specified	
	total		
Cause of death specified			1
*Morbidity (%)	major	minor	1
Morbidity index used	30-day	in-hospital	1
	operative	not specified	
	total		
Outpatient data included			1
*Procedure specific morbidity	*PLF		1
	*bile leakage		1

Article review	Quality score
*Definitions provided	1
Severity grading	1
Length of stay data	1
Risk factors analyzed	1
Total score	

RCT, randomized controlled trial; PLF, post-resectional liver failure; * item used for article selection.

Article inclusion for final analysis		
YES	**NO**	**MAYBE**

Details of articles selected for analysis		
Article reference check		
Age	mean ± standard deviation	median (range)
Sex	female	male
Indication for resection	primary liver tumour	transplantation
	secondary liver tumour	other
	benign disease	
Type of resection	major	minor
Duration of operation	mean ± standard deviation	median (range)
Clamping time	mean ± standard deviation	median (range)
Blood loss	mean ± standard deviation	median (range)
Hospital stay	mean ± standard deviation	median (range)

APPENDIX 2. FORMULA USED FOR SAMPLE SIZE CALCULATION OF EACH TREATMENT ARM OF A RANDOMIZED CONTROLLED TRIAL[1].

$$N_1 = N_2 = (cz_{1-\beta} + z_{1-\alpha/2})^2 \times \frac{2\sigma^2}{(\pi_1 - \pi_2)^2}$$

in which:
N = number of patients needed per treatment arm
π_1 = incidence in control condition
π_2 = incidence in intervention condition
σ^2 = pooled variance = π (1-π), with $\pi = (\pi_1 + \pi_2)/2$
$c = \sqrt{\{[\pi_1 (1- \pi_1)+ \pi_2 (1- \pi_2)] / 2\sigma^2\}}$*
$z_{1-\beta}$ = B-term
$z_{1-\alpha/2}$ = A-term

---

* $c < 1$ always and $c \approx 1$ usually, so $c = 1$ is an acceptable simplification

APPENDIX 3. OVERVIEW OF 19 ARTICLES WITH OVERLAPPING DATA AND THEIR METHODOLOGICAL QUALITY SCORE.

No	Author	Year	Journal	*N* patients	Quality score
1	Arru *	2007	Am Surg	100	11
2	Aldrighetti	2006	J Gastrointest Surg	200	9
3	Benzoni *	2007	Lagenbecks Arch Surg	287	9
4	Benzoni	2006	Hepatobiliary Pancreat Dis Int	287	9
5	Capussotti *	2006	Arch Surg	610	11
6	Capussotti	2006	Br J Surg	126	11
7	Sun *	2006	Br J Surg	120	14
8	Sun	2005	Hepatobiliary Pancreat Dis Int	146	11
9	Aldameh *	2005	J Gastrointest Surg	211	11
10	Koea	2005	New Zealand Medical J	100	14
11	Imamura *	2003	Arch Surg	915	11
12	Hasegawa	2002	Arch Surg	80	14
13	Aldrighetti *	2003	World J Surg	129	11
14	Aldrighetti	2004	Am Surg	103	10
15	Jarnagin *	2002	Ann Surg	1803	12
16	Burt	2002	Am J Surg	1165	10
17	D'Angelica	2006	World J Surg	1426	9
18	Kimura *	2006	J Surg Res	128	12
19	Kimura	2004	Am J Surg	60	12

N, number; For details on quality scoring see Appendix 1; * selected article.

The selected articles (marked with *) had a higher quality score. When articles had the same score, either the largest or latest publication was chosen. Exceptions were:

- The article by Koea and colleagues was not selected because it had a smaller patient number.
- The article by Imamura and colleagues was selected because it described a considerably larger patient number.

APPENDIX 4. REFERENCE LIST OF ARTICLES INCLUDED IN THE FINAL ANALYSIS.

No	Author	Quality score	*N* patients	Complication rate (%)				
				PLF	Intra-abdominal abscess	Bile leakage	Intra-abdominal hemorrhage	Mortality*
1	Hutchins	7	89	-	1.1	5.6	-	1.1 (op)
2	Ayav	12	236	0.8	8.5	2.1	-	2.1 (op)
3	Milićević	11	90	1.1	4.4	-	-	3.3 (op)
4	Watanabe	12	151	18.5	13.2	11.9	3.3	6.6 (op)
5	Figueras	15	300	6.0	1.0	10.7	-	2.7 (ho)
6	Arru	11	100	2.0	-	2.0	1.0	1.0 (op)
7	Pessaux	17	200	10.0	6.0	8.5	2.0	2.5 (30)
8	Herman	10	278	0.7	3.2	5.8	0.7	1.1 (op)
9	Evrard	12	80	1.3	1.3	5.0	3.8	1.3 (op)
10	Benzoni	9	287	9.1	11.8	2.8	4.9	4.5 (ho)
11	Kwon	11	178	1.1	5.6	8.4	3.4	1.1 (op)
12	Capussotti	11	610	-	-	3.6	-	2.5 (ho)
13	Saiura	11	60	-	-	6.7	1.7	0.0 (ho)
14	Sawada	9	91	-	-	4.4	-	0.0 (op)
15	Sun	14	120	1.7	3.3	0.0	0.8	0.8 (ho)
16	Lu	12	462	1.7	1.7	1.3	0.9	0.4 (ho)
17	Wu	14	214	-	6.5	4.7	-	0.0 (ho)
18	Kim	15	60	1.7	3.3	1.7	6.7	0.0 (op)
19	Torzilli	13	58	-	-	0.0	-	0.0 (30)
20	Aldameh	11	211	3.8	1.9	3.3	-	1.4 (op)
21	Schindl	9	104	12.5	-	5.8	1.9	1.9 (op)
22	Gruttadauria	9	149	2.7	3.4	10.1	-	3.4 (op)
23	Poon	11	1 222	3.8	2.7	3.1	1.6	4.9 (ho)
24	Imamura	11	1 056	0.1	8.2	9.2	0.9	0.0 (op)
25	Aldrighetti	11	129	3.9	-	3.9	0.8	0.8 (30)
26	Yanaga	11	60	1.7	8.3	6.7	-	0.0 (ho)
27	Bober	9	99	1.0	3.0	2.0	5.1	0.0 (op)
28	Muratore	13	53	1.9	13.2	-	1.9	0.0 (op)
29	Jarnagin	12	1 803	5.5	6.1	2.6	1.0	3.1 (op)
30	Kim	13	66	3.0	4.5	4.5	4.5	1.5 (ho)
31	Arita	16	80	-	2.5	11.3	-	0.0 (op)
32	Kimura	12	128	4.7	21.1	-	3.1	1.6 (op)

N, number; PLF, post-resectional liver failure; -, not reported; * Mortality index: (30) for 30-day mortality, (ho) for in-hospital mortality, and (op) for operative mortality.

APPENDIX 5. REFERENCE LIST OF RANDOMIZED CONTROLLED TRIALS INCLUDED IN THE FINAL ANALYSIS.

No	Author	*N* patients	Multicentre trial	Primary endpoint	Composite endpoint	Overlapping data*
1	Beck-Schimmer	64	No	Continuous	No	
2	Jarnagin	130	No	Dichotomous	No	
3	Torzilli	76	No	Continuous	No	
4	Ko	70	No	Continuous	No	
5	Heizmann	61	No	Dichotomous	Yes	
6	Nordlinger	364	Yes	Continuous	No	
7	Yuksel	32	No	Dichotomous	Yes	
8	Kim	130	No	Continuous	No	
9	Pulitano	43	No	Continuous	No	A
10	Schmidt	20	No	Continuous	No	
11	Pulitano	73	No	Continuous	No	A
12	Campagnacci	24	No	Continuous	No	
13	Hashimoto	79	No	Continuous	No	
14	Kostopanagiotou	35	No	Continuous	No	B
15	Figueras	300	No	Dichotomous	Yes	
16	Togo	180	No	Dichotomous	Yes	
17	Lupo	50	No	Dichotomous	Yes	
18	Pessaux	200	No	Dichotomous	Yes	
19	Shi	169	No	Dichotomous	No	C
20	Smyrniotis	54	No	Continuous	No	B
21	Petrowsky	73	No	Continuous	No	
22	Sugawara	81	No	Continuous	No	
23	Liu	120	No	Dichotomous	No	D
24	Esaki	92	No	Continuous	No	
25	Saiura	60	No	Continuous	No	
26	Aldrighetti	76	No	Continuous	No	A
27	Capussotti	126	No	Continuous	No	
28	Chen	118	No	Continuous	No	
29	Wang	50	No	Continuous	No	C
30	Sun	120	No	Dichotomous	No	
31	Shao	231	Yes	Dichotomous	No	
32	Wu	214	No	Dichotomous	No	
33	Azoulay	60	No	Continuous	No	
34	Kim	60	No	Continuous	No	
35	Yoshida	49	No	Dichotomous	No	
36	Arita	80	No	Continuous	No	

No	Author	*N* patients	Multicentre trial	Primary endpoint	Composite endpoint	Overlapping data*
37	Figueras	80	No	Continuous	No	
38	Smyrniotis	82	No	Dichotomous	Yes	B
39	Kanazawa	44	No	Continuous	No	
40	Lodge	185	Yes	Dichotomous	No	
41	Frilling	121	Yes	Continuous	No	
42	Lesurtel	100	No	Continuous	No	
43	Schwartz	121	Yes	Continuous	No	
44	Li	29	No	Continuous	No	C
45	Fuster	40	No	Dichotomous	No	
46	Nuzzo	42	No	Dichotomous	Yes	
47	Liu	104	No	Dichotomous	No	D

N, number; * Capital letters refer to randomized controlled trials published by one institute using data from overlapping time periods.

APPENDIX 6. OVERVIEW OF DEFINITIONS OF CLINICAL OUTCOMES USED AS ENDPOINTS OF RANDOMIZED CONTROLLED TRIALS IN LIVER SURGERY.

Ascites (*n* = 6)

1. abdominal output > 500 mL/day of ascites that required medical treatment to be controlled[2-5]
2. abdominal drain output > 500 mL/day for more than 3 days[6]
3. continuous discharge of ascites (greater than 500 mL/day) from the drainage tube for longer than 10 days postoperatively[7]

Intra-abdominal abscess (*n* = 6)

1. according to the Centres for Disease Control criteria for surgical site infections → presence of the following findings within 30 days after surgery: inflammatory findings, such as fever and flare, drainage of pus from the incision or drain, detection of a pathogen by culture of fluid or tissue sample and fluid retention on imaging indicating the presence of pus in a deep region[8]
2. purulent discharge with positive cultures from abdominal drains placed at surgery or fluid collection requiring a drainage procedure[9, 10]
3. positive culture in the presence of clinical evidence of infection[5, 11]
4. biliary communication together with purulent drainage[12]

Bile leakage/biliary fistula (*n* = 10)

1. bilirubin concentration in the drain discharge > 10 mg/dL [171 µmol/L] for at least 3 days starting from the fifth postoperative day[13]
2. drainage of bile from the abdominal wound or drain; or intra-abdominal collection of bile confirmed at the time of re-operation or percutaneous drainage or cholangiography[14]
3. drain output > 100 mL/day for >10 days[15]
4. postoperative biliary drainage through the abdominal drains[12]
5. > 1.5 mmol/L bile salts in drained fluids[16]
6. drainage of bile from the abdominal wound or drains with bilirubin content higher than the plasma levels; or intra-abdominal collection of bile at the time of re-operation or percutaneous drainage; or cholangiographic evidence of biliary leakage[2]
7. drainage of bile from the abdominal wound and drain, showing a total bilirubin level of > 5 mg/dL [85 µmol/L] or three times the serum level in the discharge fluid; or an intra-abdominal accumulation of bile confirmed by percutaneous drainage; or cholangiographic evidence of bile leakage[8]
8. bilirubin concentration in the drainage fluid > 5 mg/dL [85 µmol/L][17]
9. total bilirubin level in drainage fluid on day 14 after surgery > 5 mg/dL [85 µmol/L][18]

10. any drainage through the catheter with a bilirubin content higher than the plasma level[4]

Post-resectional liver failure/severe hepatic dysfunction (*n* = 11)

1. serum bilirubin level > 5 mg/dL [85 µmol/L] and/or prothrombin activity < 40 per cent of normal for at least 3 postoperative days. Fatal liver failure was defined as death from irreversible hepatic dysfunction (hepatic coma, massive deterioration of blood coagulation, progressive hyperbilirubinemia) in the absence of other causes, such as sepsis[14]
2. bilirubin > 100 mg/day for > 3 days[15]
3. prothrombin time < 50 per cent of normal and/or serum bilirubin > 2.9 mg/dL [50 µmol/L] on postoperative day 5 or thereafter and/or hepatic encephalopathy[2, 4]
4. simultaneous presence of a prothrombin time < 50 per cent of normal and a serum bilirubin > 2.9 mg/dL [50 µmol/L] on day 5 after surgery[19]
5. encephalopathy, ascites (volume > 500 mL/day), prothrombin time-international normalized ratio > 1.5 on day 5 or > 2 at any moment; or bilirubin level > 3 mg/dL [51 µmol/L] on day 5[5]
6. serum bilirubin concentration > 5.0 mg/dL [85 µmol/L] or a prothrombin time < 50 per cent of normal at 3 days or more after operation[20]
7. prothrombin time < 50 per cent of normal and/or serum total bilirubin > 2.9 mg/dL [50 µmol/L] and/or hepatic encephalopathy[3]
8. serum total bilirubin concentration > 5.3 mg/dL [90 µmol/L] or prothrombin time < 30 per cent of normal level within 7 days of operation. Asterixis and alteration of consciousness not related to the effect of drugs were considered signs of liver failure, even when isolated[6]
9. elevation of serum total bilirubin > 2 mg/dL [34 µmol/L] for longer than 10 days postoperatively[7]
10. total serum bilirubin 2-times higher than its normal level and massive ascites[21]

Intra-abdominal hemorrhage (*n* = 1)

1. hemorrhage requiring re-operation[6]

Mortality (*n* = 34)

1. in-hospital death[2, 4, 6, 19-27]
   a. death during the same period of hospitalization[28]
2. operative mortality[5, 8, 14, 15, 17, 18, 21, 29-32]
   a. any death resulting from a complication during surgery[33]
3. 30-day mortality[3, 9-11, 13, 16, 19, 24, 34]
4. 60-day mortality[22, 35]

5. 90-day mortality[13, 36, 37]

Wound infection (*n* = 4)

1. according to the criteria of Centres for Disease Control for surgical cite infections → the presence of the following findings within 30 days after surgery: inflammatory findings, such as fever and flare, drainage of pus from the incision or drain, detection of a pathogen by culture of fluid or tissue sample and fluid retention on imaging indicating the presence of pus in a deep region[8]
2. spontaneous or surgically released purulent discharge with positive cultures[9, 10]
3. positive culture of fluids in the presence of clinical evidence of infection[5]

Pneumonia (*n* = 4)

1. characteristic pulmonary infiltrate on a chest radiograph accompanied by leukocytosis[9, 10]
2. positive culture of sputum in the presence of clinical evidence of infection[5]
3. a productive cough with new lung findings of coarse crepitations, large areas of bronchial breathing, or dullness to percussion (in the absence of an effusion) with supporting chest radiographs[19]

Sepsis (*n* = 1)

1. temperature ≥ 38.5°C, a white blood cell count ≥ 10,000 cells/μL, and positive blood cultures or a documented septic focus[6]

Acute renal failure (*n* = 1)

1. serum creatinine > 1.7 mg/dL [150 μmol/L][6]

APPENDIX 7. SURVEY ON DEFINITIONS OF COMPLICATIONS OF LIVER SURGERY.

This survey consists of definitions of the most common complications of liver surgery in order to design a liver surgery specific composite endpoint (CEP). The survey is divided into two parts:

Part I

1. Definitions: the definitions were composed using definitions derived from literature. Please approve or disapprove these definitions. Whenever you disagree with a definition, please explain in the suggestion box. We would also like to ask your opinion on some controversies related to these definitions.
2. Liver surgery specific complications: you will be asked whether the complication is liver surgery specific or general.

Part II

1. Components of the liver surgery specific CEP: you will be asked to tell us which complications you would include in a liver surgery specific CEP.

Part I

Pleural effusion

Any fluid in pleural cavity proven by radiological imaging.

○ I approve this definition
○ I do not approve this definition

I disapprove this definition because: .....
.....

Discussion

1. Do you consider pleural effusion a complication when the patient is not respiratory compromised?

○ Yes
○ No

2. Do you consider pleural effusion a "normal" response to liver surgery?

○ Yes
○ No

Suggestion box: .....
.....

Pleural effusion is a:

○ Liver surgery specific complication
○ General complication

Ascites

Drainage of clear fluid in an amount greater than 500 mL/day via wound or intra-abdominal drain or radiologically proven intra-abdominal clear fluid from postoperative day 3 onwards.

○ I approve this definition
○ I do not approve this definition

I disapprove this definition because: .....
.....

Discussion

1. Do you think "clear fluid" should be divided into transudate or exudate?

○ Yes
○ No

2. Do you check the nature of the fluid biochemically in your daily practice?

○ Yes
○ No

Suggestion box: .....
.....

Ascites is a:

○ Liver surgery specific complication
○ General complication

Wound infection[38]

Criteria for defining a surgical site infection (SSI):

– Superficial incisional SSI: Infection occurs within 30 days after the operation and infection involves only skin or subcutaneous tissue of the incision and at least one of the following:
  1. Purulent drainage, with or without laboratory confirmation, from the superficial incision.
  2. Organisms isolated from an aseptically obtained culture of fluid or tissue from the superficial incision.
  3. At least one of the following signs or symptoms of infection: pain or tenderness, localized swelling, redness, or heat, and superficial incision is deliberately opened by surgeon, unless incision is culture-negative.

4. Diagnosis of superficial incisional SSI by the surgeon or attending physician.

– Deep incisional SSI: Infection occurs within 30 days after the operation and the infection appears to be related to the operation and infection involves deep soft tissues of the incision and at least one of the following:
  1. Purulent drainage from the deep incision but not from the organ/space component of the surgical site.
  2. A deep incision spontaneously dehisces or is deliberately opened by a surgeon when the patient has at least one of the following signs or symptoms: fever (temperature of more than 38°C), localized pain, or tenderness, unless site is culture-negative.
  3. An abscess or other evidence of infection involving the deep incision is found on direct examination, during re-operation, or by histopathological or radiological examination.
  4. Diagnosis of a deep incisional SSI by a surgeon or attending physician.

○ I approve this definition
○ I do not approve this definition

I disapprove this definition because: .....
.....

Wound infection is a:
○ Liver surgery specific complication
○ General complication

Intra-abdominal abscess

Drainage of any quantity of purulent fluid from the abdominal drain; collection of pus in the abdominal cavity at the time of re-operation or percutaneous drainage. Fluid in drain or intra-abdominal collection should be culture-positive.

○ I approve this definition
○ I do not approve this definition

I disapprove this definition because: .....
.....

Discussion

1. Do you agree the fluid should be culture-positive?
○ Yes
○ No

Suggestion box: .....
.....

Intra-abdominal abscess is a:

○ Liver surgery specific complication

○ General complication

Bile leakage

Drainage of any quantity of bile from the abdominal wound or drains at least 24hr postoperatively; intra-abdominal collection of bile at the time of re-operation or percutaneous drainage; cholangiographic evidence of contrast leakage. Fluid in drain or intra-abdominal collection should have a bilirubin content at least twice as high as bilirubin plasma concentration.

○ I approve this definition

○ I do not approve this definition

I disapprove this definition because: .....

.....

Discussion

1. Is the addition "at least 24hr postoperatively" of extra value in this definition?

○ Yes

○ No

2. Do you think biochemical evidence of bilirubin in the drain or intra-abdominal collection is a prerequisite to meet the definition of bile leakage?

○ Yes

○ No

3. Do you check the presence of bilirubin in drain or intra-abdominal fluid in your daily practice when you suspect a bile leakage? If so, please write down in the suggestion box which bilirubin level is considered to reflect bile leakage.

○ Yes

○ No

Suggestion box: .....

.....

Bile leakage is a:

○ Liver surgery specific complication

○ General complication

Sepsis[39]

The clinical syndrome defined by the presence of both infection and a systemic inflammatory response.

– Infection: pathological process caused by invasion of normally sterile tissue or fluid or body cavity by pathogenic or potentially pathogenic micro-organisms.
– Systemic inflammatory response syndrome (at least 2 criteria must be met):
  1. Body temperature higher than 38°C or lower than 36°C
  2. Heart rate higher than 90/min
  3. Hyperventilation evidenced by respiratory rate higher than 20/min or arterial carbon dioxide partial pressure lower than 32 mmHg
  4. White blood cell count higher than 12,000 cells/μL or lower than 4,000 cells/μL

○ I approve this definition
○ I do not approve this definition

I disapprove this definition because: .....
.....

Sepsis is a:

○ Liver surgery specific complication
○ General complication

Post-resectional liver failure

Failure of one or more of the hepatic excretory and synthetic functions that include hyperbilirubinemia > 50 μmol/L (equivalent to 2.9 mg/dL), prothrombin index (PT) < 50 per cent (equal to an international standardized ratio > 1.7) and/or different grades of hepatic encephalopathy (HE) from postoperative day 3 onwards or at death.

Moderate post-resectional liver failure is defined when:

a. PT index is decreased and
b. hyperbilirubinemia is present and
c. HE is absent

Severe post-resectional liver failure is defined when:

a. PT index is decreased and
b. hyperbilirubinemia is present and
c. HE is present

○ I approve this definition
○ I do not approve this definition

I disapprove this definition because: .....
.....

Discussion

1. Do you think "from postoperative day 3 onwards" is of extra value in this definition?

○ Yes
○ No

2. Do you think other parameters reflecting liver synthetic or excretory function should be included in this definition? If yes, please insert these parameters in the suggestion box.

○ Yes

○ No

Suggestion box: .....

.....

Post-resectional liver failure is a:

○ Liver surgery specific complication

○ General complication

Pneumonia[38]

Clinical entity meeting one of the following criteria:

1. Rales or dullness to percussion on physical examination of the chest and any of the following:
   a. New onset of purulent sputum or change in character of sputum.
   b. Organisms isolated from blood culture.
   c. Isolation of pathogens from specimen obtained by transtracheal aspirate, bronchial brushing, or biopsy.
2. Chest radiographic examination shows new or progressive infiltrate, consolidation, cavitation, or pleural effusion and any of the following:
   a. New onset of purulent sputum or change in character of sputum.
   b. Organisms isolated from blood culture.
   c. Isolation of pathogens from specimen obtained by transtracheal aspirate, bronchial brushing, or biopsy.
   d. Isolation of virus or detection of viral antigen in respiratory secretions.
   e. Diagnostic single antibody titer (IgM) or fourfold increase in paired serum samples (IgG) for pathogen.
   f. Histopathological evidence of pneumonia.

○ I approve this definition

○ I do not approve this definition

I disapprove this definition because: .....

.....

Pneumonia is a:

○ Liver surgery specific complication

○ General complication

Intra-abdominal bleeding

Leakage of blood via the abdominal drain with hemodynamic instability or asymptomatic bleeding that causes a collection of blood in proximity of the liver (hematoma) and that can be diagnosed radiologically.

○ I approve this definition
○ I do not approve this definition

I disapprove this definition because: .....
.....

Discussion

1. Do you think we should specify the quantity of blood? If so please notify in the suggestion box how much blood you think will qualify for bleeding.

○ Yes
○ No

2. Do you think the hematoma should be radiologically proven?

○ Yes
○ No

Suggestion box: .....
.....

Intra-abdominal bleeding is a:

○ Liver surgery specific complication
○ General complication

Acute renal failure[40]

An increase in serum creatinine of 0.5 mg/dL (44.2 µmol/L) if baseline level is less than 2.5 mg/dL (221 µmol/L) or an increase in serum creatinine by more than 20% if baseline level is more than 2.5 mg/dL (221 µmol/L).

○ I approve this definition
○ I do not approve this definition

I disapprove this definition because: .....
.....

Acute renal failure is a:

○ Liver surgery specific complication
○ General complication

Blood transfusion

Requirement of any quantity of packed cells during surgery or after surgery.

○ I approve this definition

○ I do not approve this definition

I disapprove this definition because: .....

.....

Discussion

1. Do you think we should quantify the blood loss? If yes, please specify in the suggestion box what quantity would be required to complete the definition.

○ Yes

○ No

2. Do you agree we should extend the period of blood loss beyond the surgery time? If yes, please specify in the suggestion box how long blood loss should be recorded.

○ Yes

○ No

Suggestion box: .....

.....

Requirement of blood transfusion is a:

○ Liver surgery specific complication

○ General complication

Operative mortality

Death of a patient directly related to liver surgery.

○ I approve this definition

○ I do not approve this definition

I disapprove this definition because: .....

.....

Part II

Now, we would like to ask you to inform us on the complications you think should be included as components of the liver surgery specific CEP. For your information, we provide some general characteristics of CEPs[41].

1. A CEP is an outcome that captures the number of patients experiencing one or more complications.
2. The components of a CEP should be ascertainable without bias.

3. The components of the CEP must be associated with the primary objective of a clinical trial (e.g. the effect of an intervention on surgery-related mortality and morbidity).
4. The validity of a CEP depends on similarity in
   - clinical importance of the components from the patient's perspective (preferably: equal or nearly equal severity grade).
   - effect of the intervention on the incidence of the component (preferably: equal or nearly equal effect size).
   - incidence rate of each component (preferably: equal or nearly equal incidence rate).

Which complications depicted in the Table should be included in the liver surgery specific CEP according to you?

	Yes	No
Pleural effusion		
Ascites		
Wound infection		
Intra-abdominal abscess		
Bile leakage		
Sepsis		
Post-resectional liver failure		
Pneumonia		
Intra-abdominal bleeding		
Acute renal failure		
Requirement of blood transfusion		
Operative mortality		

Would you be interested in using a liver surgery specific CEP as primary endpoint of a randomized controlled trial?

○ Yes
○ No

Would you be willing to share your data from a previously conducted randomized controlled trial in liver surgery for determination of the current incidence rate and validation of the liver surgery specific CEP?

○ Yes, contact me for further details
○ No, I'm not interested in sharing my data

APPENDIX 8. OVERVIEW OF RESPONDENTS TO THE WEB-BASED SURVEY.

- Prof. dr. R Adam, Centre Hépato-Biliaire, Hôpital Paul Brousse, Villejuif, France
- Drs. M de Boer, Department of Surgery, University Medical Centre Groningen, Groningen, the Netherlands
- Prof. dr. I Borel Rinkes, Department of Surgery, University Medical Centre Utrecht, Utrecht, the Netherlands
- Prof. dr. O Busch, Department of Surgery, Academic Medical Centre, Amsterdam, the Netherlands
- Prof. dr. L Capussotti, Department of Surgery, Ospedale Mauriziano Umberto I, Torino, Italy
- Prof. dr. D Castaing, Centre Hépato-Biliaire, Hôpital Paul Brousse, Villejuif, France
- Prof. dr. PA Clavien, Department of Visceral and Transplantation Surgery, Swiss HPB Centre, Zürich, Switzerland
- Prof. dr. B Davidson, Department of Surgery, Royal Free Hospital, University College London, London, UK
- Prof. dr. C Dejong, Department of Surgery, Maastricht University Medical Centre, Maastricht, the Netherlands
- Prof. dr. J Figueras, Department of Surgery, Josep Trueta Hospital, Girona, Spain
- Dr. Y Fong, Division of Hepatopancreatobiliary Surgery, Memorial Sloan-Kettering Cancer Centre, New York, USA
- Prof. dr. O Garden, Department of Surgery, Royal Infirmary of Edinburgh, Edinburgh, UK
- Prof. dr. D Gouma, Department of Surgery, Academic Medical Centre, Amsterdam, the Netherlands
- Prof. dr. N Habib, Department of Surgery and Cancer, Imperial College, London, UK
- Prof. dr. R Hillegersberg, Department of Surgery, University Medical Centre Utrecht, Utrecht, the Netherlands
- Prof. dr. W Jarnagin, Division of Hepatopancreatobiliary Surgery, Memorial Sloan-Kettering Cancer Centre, New York, USA
- Dr. J Klaase, Department of Surgery, Medical Spectrum Twente, Enschede, the Netherlands
- Prof. dr. P Lai, Department of Surgery, Prince of Wales Hospital, Chinese University of Hong Kong, Shatin, Hong Kong
- Prof. dr. H Lang, Department of General and Abdominal Surgery, Johannes Gutenberg-University Hospital, Mainz, Germany
- Prof. dr. W Lau, Faculty of Medicine, Chinese University of Hong Kong, Hong Kong, China

- Dr. E Manusama, Department of Surgery, Medical Centre Leeuwarden, Leeuwarden, the Netherlands
- Prof. dr. P Neuhaus, Department of General, Visceral, and Transplantation Surgery, Charité, Campus Virchow-Klinikum, Humboldt University, Berlin, Germany
- Prof. dr. R Parks, Department of Surgery, Royal Infirmary of Edinburgh, Edinburgh, UK
- Prof. dr. R Porte, Department of Surgery, University Medical Centre Groningen, Groningen, the Netherlands.
- Prof. dr. A Revhaug, Surgical Research Laboratory, Institute of Clinical Medicine, University of Tromsö, Tromsö, Norway
- Dr. A Rijken, Department of Surgery, Amphia Hospital, Breda, the Netherlands
- Dr. R Roumen, Department of Surgery, Maxima Medical Centre Veldhoven, Veldhoven, the Netherlands
- Dr. J van der Sijp, Department of Surgery, Medical Centre Haaglanden, Den Haag, the Netherlands
- Dr. C Verhoef, Department of Hepatobiliary and Transplantation Surgery, Erasmus Medical Centre, Rotterdam, the Netherlands
- Prof. dr. S Wigmore, Department of Surgery, Royal Infirmary of Edinburgh, Edinburgh, UK
- Prof. dr. J de Wilt, Department of Surgery, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands

APPENDIX 9. PRIMERS USED FOR QUANTITATIVE POLYMERASE CHAIN REACTION.

Name	Forward	Reverse
PPIA*	CTCGAATAAGTTTGACTTGTGTTT	CTAGGCATGGGAGGGAACA
IL-1β	CTGAGCTCGCCAGTGAAATG	TTTAGGGCCATCAGCTTCAAA
IL-6	TCCAGGAGCCCAGCTATGAA	GAGCAGCCCCAGGGAGAA
IL-8	CTGGCCGTGGCTCTCTTG	TTAGCACTCCTTGGCAAAACTG
ICAM-1	CTGAGCAATGTGCAAGAAGATAGC	CCCGTTCTGGAGTCCAGTACA
VCAM-1	GCCGATCACAGTCAAGTGTTCA	CATGAGATGATCTCCTTTCAGTAAGTCT

* House-keeping gene Cyclo-A; IL, interleukin; ICAM-1, intercellular adhesion molecule; VCAM-1, vascular cell adhesion molecule.

APPENDIX 10. HEPATIC DAMAGE IN PATIENTS UNDERGOING LIVER SURGERY WITHOUT AND WITH INTERMITTENT PRINGLE MANEUVER.

	Control group	Intervention groups	
	noIPM (*n* = 10)	15 IPM *(n* = 10*)*	30IPM *(n* = 10*)*
L-FABP (ng/mL)			
Baseline	24 ± 6	24 ± 8	28 ± 4
Before mobilization	409 ± 206	179 ± 65	316 ± 162
Before transection	1102 ± 111*	1229 ± 279*	1573 ± 516*
30′ cumulative ischemia	920 ± 157*	762 ± 170	543 ± 69*
5′ reperfusion	880 ± 120*	1209 ± 262	2303 ± 602*
After transection	877 ± 109*	1853 ± 708*	3662 ± 1355
8hr after start	366 ± 168	791 ± 221*	485 ± 66*
POD1	146 ± 28	370 ± 109	496 ± 108
POD2	59 ± 15	137 ± 44	234 ± 89
POD3	25 ± 7	38 ± 15	42 ± 9
ALAT (IU/L)			
Baseline	34 ± 9	47 ± 10	27 ± 5
Before mobilization	38 ± 9	48 ± 9	34 ± 7
Before transection	119 ± 18*	151 ± 17*	132 ± 24*
30′ cumulative ischemia	198 ± 42*	159 ± 27	156 ± 27*
5′ reperfusion	168 ± 44	179 ± 29	202 ± 40*
After transection	201 ± 50	280 ± 80	452 ± 127
8hr after start	289 ± 78	577 ± 242*	701 ± 245
POD1	328 ± 67*	708 ± 244	697 ± 172*
POD2	265 ± 65	384 ± 94	859 ± 433
POD3	208 ± 35	449 ± 128	609 ± 232
ASAT (IU/L)			
Baseline	55 ± 22	38 ± 8	24 ± 5
Before mobilization	44 ± 13	42 ± 7	30 ± 6
Before transection	138 ± 20	169 ± 22*	155 ± 27*
30′ ischemia	199 ± 37	189 ± 31*	196 ± 33*
5′ reperfusion	198 ± 44	210 ± 33*	246 ± 54*
After transection	225 ± 50	296 ± 72*	510 ± 129
8hr after start	255 ± 35*	590 ± 232*	861 ± 275
POD1	391 ± 89*	756 ± 260*	837 ± 219*
POD2	202 ± 26	276 ± 46*	852 ± 262
POD3	112 ± 16	218 ± 52	236 ± 59

Values are mean ± standard error; 15IPM, 15 minutes intermittent Pringle maneuver; 30IPM, 30 minutes intermittent Pringle maneuver; noIPM, no intermittent Pringle maneuver; L-FABP, liver fatty acid-binding protein; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; POD, postoperative day; * repeated measures two-way ANOVA with post-hoc paired sample t-test versus baseline: $p<0.050$.

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Scientific output

PUBLISHED PAPERS

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SUBMITTED MANUSCRIPTS

23. Pessaux P, **van den Broek MA**, Marzano E, Olde Damink SW, Piardi T, Dejong CH, Ntourakis D, van Dam RM. Identification and validation of risk factors for postoperative infectious complications following hepatectomy. *Submitted with revisions.*
24. Bloemen JG, **van den Broek MA**, Venema K, Buurman WA, Dejong CH. Randomized placebo controlled study of the effects of butyrate enemas on systemic concentrations and splanchnic fluxes of short chain fatty acids. *Submitted.*
25. Bloemen JG, van der Vorst JR, **van den Broek MA**, Venema K, Buurman WA, Dejong CH. Effects of liver resection on hepatic short chain fatty acid handling in humans. *Submitted.*

MANUSCRIPTS IN PREPARATION

26. van Dam RM, Lodewick TM, **van den Broek MA**, de Jong MC, Greve JW, Jansen RL, Bemelmans MH, Olde Damink SW, Dejong CH. Outcomes of contemporary versus classical indications for patients undergoing liver surgery for colorectal cancer liver metastases.
27. **van den Broek MA**, Olde Damink SW, Adamzik M, Dejong CH, Broelsch CE, Paul A, Malagó M, Saner FH. Gene polymorphisms and the risk of infection and infection-related mortality in living donor liver transplant recipients.

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En dan vanzelfsprekend mijn onderzoekscollega's. Johanne (beter bekend als mijn zeer begaafde tweelingzus genaamd JB, bloempje, jut, snap, knabbel, stClaire of Jozef): de humane leverchirurgie studies hebben we samen verricht onder het motto "zonder dalen, geen pieken". Wij weten wat reanimeren is… Maar naast de wetenschap waren daar ook bieren in de Thembi's, (après-) skiën in Sankt Anton, party(crash)en in Maastricht, flaneren door Londen en feesten in Athene. Zowel collega's als echte vrienden. Het blijft jammer dat we niet in driedelig wit door hetzelfde ziekenhuis struinen! Gelukkig bied je altijd een luisterend oor als ik in het Westen weer eens bezig ben een wetenschappelijke of klinische uitdaging te overwinnen. Wie anders dan jij zou mijn "ene" paranimf moeten zijn?

Mijn roomies van de 3/1: allereerst Ruben, studie- en kamergenoot van het eerste uur. Vier jaar lang hebben we, eerst zij aan zij en daarna back to back, gewerkt aan de totstandkoming van onze pareltjes. Ook al verschillen onze karakters totaal, ik kan toch met recht zeggen dat er maar weinigen waren die mij zo goed konden peilen als jij. Ik heb ontzettend veel respect voor jouw onverwoestbare doorzettingsvermogen en

ongelooflijk stabiel neutrale pH. Om onze samenwerking in stijl te bezegelen: daanke jong en veer zien us!

Kirsten alias Frau Huntjens: we are sure we want to close SPSS! Wat hebben we ellenlang zitten zwoegen op onze databases en bijna-voltooide-maar-het-moet-toch-echt-nog-net-even-anders manuscripten. Gedeelde smart was halve smart… Maar ook je steun en motiverende woorden tijdens de afrondende fase van mijn proefschrift, toen we allebei al in de Randstad woonden en werkten, waren voor mij erg belangrijk. Wat fijn dat we de vrijdagmiddagborrel gewoon voort kunnen zetten in onze nieuwe Thembi's in de Residentie!

ArgiNina, Lianne en Mascha, onze vele gezamenlijke kopjes koffie in ons bijzondere kantoor op de 3 zorgden voor de onmisbare ontspanning na (maar vaak ook voor) wetenschappelijke inspanning. Simon, ik ben je dankbaar voor je hulp bij het verzamelen, beheren en analyseren van al het patiëntenmateriaal. Mechteld, jij nam mijn bureau en plaats in het MUMC HPB team met veel succes over. Jammer dat we niet aan samenwerken zijn toegekomen, want onze bezoekjes aan de IHPBA congressen in Buenos Aires en Kaapstad smaakten zeker naar meer!

Tot slot ben ik natuurlijk ook alle collega's van de 5, in het bijzonder de vaste steunpilaren Annemarie, Bas, Hans, Kaatje, Mo, Sander en (chef reservebank) Tim, en de studenten die hebben bijgedragen aan de datacollectie van een aantal manuscripten, ontzettend dankbaar.

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En natuurlijk mijn Leiderdorpse collega's, het was spannend om in een nieuwe regio aan mijn opleiding tot chirurg te beginnen. Mede door de geweldige sfeer in de assistenten- en opleidersgroep voelde ik me vanaf de eerste dag thuis in het Rijnland Ziekenhuis. Ongelooflijk bedankt voor de klinische tips and tricks, de duwtjes in de rug op momenten dat ik ze nodig had en, niet te vergeten, de mooie borrels, BBQ's en feestjes!

Wie en waar zou ik zijn zonder mijn vrienden? Het "project-not-to-be-named" is eindelijk af! Er is weer tijd voor leuke dingen: winen en dinen, hardlopen, shoppen, flaneren langs het strand, reizen en ga zo maar door.

Klaar en Monique, we zijn met z'n drieën aan onze studententijd in Maastricht begonnen. We hebben samen, zowel letterlijk als figuurlijk, vele hoogtepunten beleefd en ik hoop dat er ook nog vele zullen volgen. Klaar en Hans, ik geniet van de eindeloze diners en logeerpartijtjes in de wereldstad Vleuten. Fijn om te weten dat ik altijd bij jullie kan aankloppen! Monique, jouw humor en nuchtere blik op het leven brengen mij altijd weer met beide benen op de grond en zijn daarom erg belangrijk voor mij.

Nanette, DB'er in hart en nieren, mijn dank voor alle goed-foute adviezen, shop-till-we-drop momenten, relatieadviezen, verkleedpartijtjes, springfoto's, en TY beautytips is onbeschrijflijk groot! En wat fijn dat ik ook altijd voor "serious business" bij je terecht kan.

Saskia en Michiel, ik heb genoten van jullie Bourgondische levensinstelling. Helaas zijn mijn bezoekjes aan het Zuiden nog maar schaars, maar Vastelaovend kan nergens anders gevierd worden dan in Maastricht.

Vrienden uit de Nationale DenkTank 2008: onder het motto "kennis maken zonder kaders" hebben we een inspirerende en leerzame tijd beleefd in Amsterdam. Vaak denk ik met een glimlach terug aan onze MECE issue trees, out of the box brainstorm sessies en gelikte PowerPoint presentaties. Dank, dank, dank!

Last but not least, mijn familie. Lieve Thijs en Karin, we hebben door de jaren heen veel mooie avonturen beleefd. Als klap op de vuurpijl waren daar onze tripjes naar Argentinië en Andalusië, mijn ceremoniemeesterschap op jullie bruiloft en de geboorte van Tiemen. Ik hoop dat er in de toekomst nog veel meer van dit soort onvergetelijke momenten zullen volgen! Karin, ik prijs me gelukkig met zo'n lieve en betrokken schoonzus. Thijs, jij bent echt mijn Grote Broer. Jouw wijze raad heeft mij op cruciale momenten de juiste weg gewezen. Ik bewonder je positieve levensinstelling, eloquentie, vastberadenheid en onnavolgbare logica! Met jou als "andere" paranimf kan er echt niets meer mis gaan, wat een fijn idee!

Papa en mama, jullie vormden de basis van wie ik nu ben. Door jullie opvoeding heb ik geleerd dat het leven een vat vol keuzes is. Al van jongs af aan gaven jullie mij de ruimte om deze keuzes zélf te maken en, op die manier, mijn dromen te leven. Jullie rotsvaste vertrouwen en geloof in de juistheid van mijn keuzes (en de wetenschap dat jullie er altijd zullen zijn als zij toch wat minder gunstig uitvallen) zijn voor mij erg belangrijk. Eigenlijk geldt voor jullie wat we vroeger vaak samen zongen: "Woorden schieten toch tekort…"! Ik ben enorm trots dat jullie mijn ouders zijn!

Curriculum vitae

Maartje van den Broek was born in Breda, the Netherlands, on 7 March 1981. After graduating from secondary school (Sint Oelbert Gymnasium, Oosterhout, 1999, *cum laude*), she started her medical training at Maastricht University. As a medical student, she spent a semester at the Departments of Primary Health Care and Paediatrics of BP Koirala Institute of Health Sciences, Nepal. After obtaining her master's degree (2003, *cum laude*), she started her internships. During her internships, she performed a research elective at the Department of Surgery of Maastricht University (prof. dr. WA Buurman), where her interest in performing translational research in surgery was born. She graduated from medical school in 2005 (*cum laude*). Subsequent to graduating, she worked as a surgical resident at Atrium Medical Centre Parkstad (dr. RJ Welten).

In September 2006, she started her PhD project at the Department of Surgery of Maastricht University Medical Centre, which resulted in the present thesis (prof. dr. CH Dejong and dr. SW Olde Damink). During her PhD project, she worked as a research assistant at Essen University Hospital, Germany (prof. dr. CE Broelsch) and University College London Hospitals, United Kingdom (prof. dr. M Malagó). She was awarded a Gastrostart grant from the Netherlands Society of Gastroenterology, a research grant from Stichting De Drie Lichten and travel grants from the British Association for the Study of the Liver, the United European Gastroenterology Organization and the Netherlands Society of Gastroenterology. The scientific work described in this thesis was presented at multiple national and international conferences.

In addition to her work as a PhD student, she was a member of the Pélerin scientific committee, responsible for the organization of the annual scientific symposium for residents of Maastricht University Medical Centre. She also participated in the Dutch National ThinkTank 2008, which resulted in the publication of a final report entitled "Healthy together! Tips to motivate teenagers into choosing a healthy lifestyle". Currently, she is a member of the Young Society, which is a subsidiary of the Royal Holland Society of Sciences and Humanities.

In July 2010, she started her surgical training at the Department of Surgery of Rijnland Hospital (dr. SA da Costa and dr. AM Zeillemaker), which is part of the educational region of Leiden University Medical Centre (prof. dr. JF Hamming).