Chemotherapy-associated liver injury

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Chemotherapy-associated liver injury:

mechanisms and impact on clinical outcome

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Chemotherapy-associated liver injury:

mechanisms and impact on clinical outcome

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Chapter 1

General introduction,
aims and outline of the thesis

Introduction

By affecting over 1.3 million patients annually,¹ colorectal cancer is the third most common cancer worldwide. Approximately half of these patients develop colorectal liver metastases (CRLM).^{2,3} Hepatic resection provides the best prospect of cure, but only up to 30 per cent of patients with liver metastases are initially eligible for hepatectomy.^{3,4} Neoadjuvant chemotherapy may reduce tumour burden and facilitate future partial liver resection for those with tumours deemed unresectable.^{5,6} Usage of combinations of chemotherapeutic agents, such as oxaliplatin and irinotecan, has increased tumour response rates remarkably from 24 per cent to up to 70 per cent, compared to single treatment with 5-fluorouracil.^{7,8}

The beneficial effect from systemic chemotherapy has been proven in randomized control trials. Despite that, the undesired liver parenchymal damage caused by chemotherapy, collectively referred to as chemotherapy-associated liver injury (CALI), is not negligible. For instance, oxaliplatin-based treatments increase the occurrence of sinusoidal obstruction syndrome (SOS)^{9,10} and nodular regenerative hyperplasia,¹⁰⁻¹² treatment with a combination of 5-fluorouracil and leucovorin is associated with the development of steatosis,¹³ whereas irinotecan-based regimens appear to be related to steatohepatitis.¹⁴ Once occurred, CALI persists for a long period even after cessation of chemotherapy. Vigano et al. have recently shown that SOS and nodular regeneration may regress only after nine months without chemotherapy, whereas steatosis and steatohepatitis persist.¹⁵

SOS, previously known as veno-occlusive disease, is a syndrome characterized by injury of the sinusoidal endothelial cells, sinusoidal dilatation, peliosis, venular lesions and fibrosis; sinusoidal dilatation is the hallmark feature of SOS.^{9,10} A grading system developed by Rubbia-Brandt et al. allows pathologists to directly score sinusoidal dilatation, and is routinely used for stipulating severity of SOS.^{9,10} As a result of SOS, the hepatic microcirculation is disorganized with hyper-perfused zones intermingled with hypo-perfused zones,¹⁶ which causes regenerative hepatocyte plates intermingled with atrophic plates. Secondary to sinusoidal injury, nodular regeneration is likely a regenerative response to sinusoidal injury, and characterized by diffuse transformation of regular hepatic parenchyma into regenerative nodules, compressing the surrounding parenchyma leading to appearance of atrophic hepatocytic plates.¹⁰

Steatosis and steatohepatitis, which are characterized by dysregulation of hepatic fat accumulation with or without neutrophil infiltration, are also part of a broad range of

liver injuries that may be caused by specific drugs.^{13,14} The grading system of Kleiner et al. distinguishes histological features of steatosis, lobular inflammation, hepatocellular ballooning, and fibrosis, allowing pathologists to quantify the severity of steatosis and steatohepatitis.¹⁷ Alternatively, a novel scoring system (steatosis, activity, and fibrosis, [SAF]) with supposedly reduced inter-observer variation has been recently proposed to evaluate severity of steatohepatitis.¹⁸

CALI and short-term outcome after partial hepatectomy for CRLM

An overview of research themes in the current thesis is presented in Figure 1.1 (dashed lines). The potential downstream side-effects of CALI on clinical outcome after partial hepatectomy are an active area of research surrounded with controversy. Despite certain studies claiming that the presence of CALI is associated with increased postoperative morbidity and mortality,¹⁹⁻²⁴ others could not reproduce this.²⁵⁻²⁹ The presence of postoperative infectious complications, particularly surgical site infections, remains a crucial clinical concern that is associated with increased morbidity and mortality rates,³⁰ prolonged length of hospital stay,³¹ increased healthcare cost,^{32,33} and even decreased long-term survival.^{30,34} Regardless of numerous advances made into practice to prevent infection, surgical site infections remain the second most common healthcare-related infection after respiratory tract infection.³⁵ Nevertheless, our understanding of the impact of CALI on infectious complications after partial hepatectomy is poor, and the relation between CALI and postoperative morbidity and mortality remains unclear (Figure 1.1, blue symbols).

CALI, tumour response, and long-term survival after partial hepatectomy for CRLM

Apart from exploring the relation between CALI and postoperative short-term outcome, it is of importance to understand whether CALI has an impact on long-term survival. It has been shown by our group³⁶ and subsequently by others³⁷ that CALI is related to poor tumour response, which is expressed as tumour regression grade, to neoadjuvant chemotherapy (Figure 1.1, green symbols). Since CALI impairs tumour response, and a good tumour response facilitates future hepatic resection (the best cure for CRLM),^{5,6} it is reasonable to presume that the presence of CALI may be related to decreased survival. However, studies into this area are few. Although one study claims that

chemotherapy-associated sinusoidal dilatation is associated with shortened survival,³⁸ a second could not reproduce this.³⁷

Figure 1.1 Research themes regarding chemotherapy-associated liver injury in this thesis. TRG, tumour regression grade; CALI, chemotherapy-associated liver injury; solid lines indicate that the relation between two connected components is known in literature; dashed lines indicate that the relation between two connected components is controversial or unknown in literature; arrows indicate a likely cause-effect relationship.

MicroRNAs: regulators of gene expression in CALI?

Although CALI is likely to be of significant clinical importance, there is a lack of biomarkers that predict its occurrence, and there are currently no options to treat CALI. Furthermore, the molecular mechanisms underlying its development are still mostly unknown. These gaps in our current knowledge might be filled by studying the link between CALI and so-called microRNAs (Figure 1.1, yellow symbols), small non-coding RNA molecules that function as regulators of gene expression through translational repression and/or mRNA cleavage.³⁹ Indeed, the concentration of several microRNAs has been found to be enriched in the serum of patients with acetaminophen-induced liver injury.⁴⁰ Furthermore, specific circulating microRNAs are known to represent reliable biomarkers for predicting tumour response to chemotherapy and prognosis.^{41,42} Importantly, treating hepatitis C by targeting a specific microRNA involved in its pathogenesis has been shown to be feasible and effective.⁴³

Complement activation: the potential role of inflammation in CALI

The liver contains numerous innate and adaptive immune cells, and represents a critical component in immune surveillance.⁴⁴ As an important element of the innate immune system, the complement system mediates the regulation of immune responses to tissue injury and the initiation of inflammatory reactions.⁴⁵ Pathophysiological processes leading to CALI involve an activation of the hepatic acute phase response.⁴⁶ The acute phase response comprises a series of prompt immune responses to tissue injury,⁴⁷ and may include activation of the complement pathways.^{48,49} Hepatic parenchymal injury as occurs in CALI may lead to the formation of C3a, which is cleaved from the central complement factor C3, and which promotes inflammation by recruiting neutrophils. The recruitment of these neutrophils has been shown to be essential for the development of nonalcoholic steatohepatitis, another form of sterile liver damage.⁵⁰ Furthermore, C3a is rapidly cleaved by carboxypeptidases to generate acylation stimulating protein, a peptide that regulates triglyceride metabolism.⁵¹ As stated above, excessive accumulation of triglycerides in hepatocytes, or hepatic steatosis,⁵² is another important component of CALI and a central feature of steatohepatitis. Continued complement activation may result in the formation of so-called C5 convertase, which cleaves complement factor C5 into C5b and C5a. Apart from promoting inflammation via attracting circulating neutrophils into the liver,⁵³ C5a has been suggested to regulate hepatic fibrosis by stimulating hepatic stellate cells⁵⁴⁻⁵⁶ and to play a role in the early stage of liver regeneration.^{57,58} Given that these (patho)physiological processes frequently occur in CALI, complement activation could play a role in its development (Figure 1.1, yellow symbols).

Aims of this thesis

The main aims of the current thesis were: **1)** to explore whether CALI (i.e. sinusoidal dilatation, nodular regeneration, steatosis, and steatohepatitis) is a risk factor for increased morbidity (e.g. liver surgery-specific morbidity and major morbidity) and mortality, as well as infectious complications after partial hepatectomy for colorectal liver metastases; **2)** to validate the relation between CALI and the tumour response to treatment, and further explore whether CALI is associated with survival after partial hepatectomy for CRLM; **3)** to obtain novel insight into the pathogenesis of CALI, in particular sinusoidal injury, by identifying hepatic microRNAs associated with the development of such lesions; **4)** to study the links between these microRNAs and tumour

recurrence as well as survival; **5**) to investigate whether complement activation plays a role in the development of CALI in patients undergoing partial hepatectomy for CRLM.

Outline of this thesis

In **Part I**, the impact of CALI (i.e. sinusoidal dilatation, nodular regeneration, steatosis, and steatohepatitis) on postoperative short-term (i.e. morbidity and mortality) and long-term (i.e. recurrence-free survival and overall survival) outcome is investigated. A systematic review with meta-analysis is described to study the impact of chemotherapy-associated sinusoidal dilatation on short-term outcome after partial hepatectomy for CRLM (**Chapter 2**). The existence of variations in study design and inclusion criteria, diverse definitions of a single endpoint, and varied endpoints under the category of morbidity as outlined in **Chapter 2** raised the awareness that an unification for statistical modelling and a consensus definition for endpoints in CALI research is needed, and this led us to conduct a systematic review with individual participant data analyses on this topic (**Chapter 3**). **Chapter 4** provides a detailed summary of the impact of CALI on postoperative infectious complications after partial hepatectomy for CRLM. Furthermore, the influence of CALI (i.e. sinusoidal dilatation, nodular regeneration, steatosis, and steatohepatitis) on tumour response to preoperative treatment, and thereafter on postoperative long-term outcome was explored. **Chapter 5** describes a study on the influence of CALI on tumour response after neoadjuvant chemotherapy, and a summary of the association between CALI and long-term survival (i.e. recurrence-free survival and overall survival) after partial hepatectomy for CRLM.

In view of the clinical observations in **Part I**, and the lack of biomarkers to predict and options to treat CALI, **Part II** focuses on exploring the mechanisms underpinning CALI. **Chapter 6** provides novel insights into the pathogenesis of CALI-related sinusoidal injury by identifying hepatic microRNAs associated with the development of such lesions. **Chapter 7** describes the relation between CALI and hepatic complement activation in patients who had undergone partial hepatectomy for CRLM.

Chapter 8 summarizes the main findings as discussed in **Part I** and **Part II** of this thesis, provides a general discussion of those findings, and describes clinical implications and future perspectives of the research performed.

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Part I

Influence of chemotherapy-associated liver injury on
outcome after partial hepatectomy for
colorectal liver metastases

Chapter 2

The influence of chemotherapy-associated sinusoidal dilatation on short-term outcome after partial hepatectomy for colorectal liver metastases: a systematic review with meta-analysis

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*Both authors contributed equally to this study

Surg Oncol 2016; 25(3):298-307

Abstract

Background

Hepatic sinusoidal dilatation (SD) is a histopathological entity that occurs in up to 75% of patients undergoing oxaliplatin-based chemotherapy for colorectal liver metastases (CRLM). The aim was to study the influence of SD on outcome after partial hepatectomy in patients with CRLM.

Methods

Medline, Embase, CENTRAL, LILACS and CINAHL were searched for studies published between 01.01.2004 and 09.06.2015 with keywords: “sinusoidal obstruction syndrome”, “hepatic veno-occlusive disease”, and “Stuart-Bras syndrome”. Studies comprising adults who underwent partial hepatectomy for CRLM with grading of SD and registration of postoperative morbidity and/or mortality were included. Risk of bias and quality of studies were evaluated with the Quality In Prognosis Studies Instrument (QUIPS) and modified GRADE framework.

Results

Search strategies produced 2007 hits from which 23 and 13 articles were extracted for qualitative and quantitative analyses, respectively. Meta-analysis on the influence of SD grade 2-3 vs. SD grade 0-1 on postoperative overall morbidity showed an odds ratio (OR) of 1.26 (95% CI 0.74-2.15, $p=0.40$), an OR of 1.03 (95% CI 0.15-6.89, $p=0.98$) for liver failure, an OR of 1.21 (95% CI 0.23-6.35, $p=0.82$) for overall mortality, and an OR of 3.52 (95% CI 0.31-39.91, $p=0.31$) for liver-related morbidity. QUIPS showed a low to high risk of bias for studies, and GRADE showed very low quality of evidence per outcome.

Conclusions

No significant effect of SD grade 2-3 on short-term outcome after partial hepatectomy was found. However, the data on which this conclusion was based were not very robust and therefore no solid conclusions could be drawn.

Introduction

Sinusoidal dilatation (SD) is a common manifestation of hepatotoxicity that occurs in patients with colorectal liver metastases (CRLM) after administration of oxaliplatin-based chemotherapy.¹⁻³ Regimens based on the platinum containing agent oxaliplatin are used extensively as neo-adjuvant therapy to downsize initially irresectable CRLM, with convincing response rates and survival outcomes.⁴⁻⁶ However, liver injury is demonstrated in over 75% of patients.^{1, 3}

SD is part of a broad range of liver injuries due to specific drugs, in conjunction with sinusoidal obstruction syndrome (SOS). SOS is macroscopically identified as 'blue liver' and microscopically characterized by injury of the sinusoidal endothelial cells (SECs), parenchymal lesions (e.g. SD and peliosis), venular lesions, and fibrosis. Various pathogenic factors have been described to contribute to these histopathological changes.⁷ Key features of oxaliplatin-induced toxicity are its reaction with reduced glutathione and F-actin depolymerization, which results in rounding up and subsequent dehiscence of SECs and obstruction of sinusoidal blood flow leading to SD and erythrocyte extravasation.^{1,8-12} Activation of hepatic stellate cells (HSC) results in neodisposition of collagen bundles in the perisinusoidal space which, in combination with subendothelial fibroblast activation in the terminal hepatic vein, leads to fibrotic venular occlusion. In the last stage of SOS, dense perivenular fibrosis is observed and hepatic vein lumina can no longer be identified.¹³

The grading system of Rubbia-Brandt et al. classifies all histological features of SOS including SD, and is routinely used for stipulating severity.^{1,2} In most studies, SD functions as the standard for liver damage. Reversibility of sinusoidal injury is discussed widely, and although human and animal models show ceasing of pathological features at repeated hepatic resection,^{2,14} other studies show persistence or progression of lesions even after cessation of chemotherapy.¹⁵

Clinical importance of SD is reflected in the development of hepatomegaly, ascites, splenomegaly, thrombocytopenia, portal hypertension, and systemic elevation of liver enzymes.¹⁶⁻¹⁹ With regard to liver surgery, a diminished preoperative functional reserve, (transient) postoperative liver failure, higher morbidity rates and a longer hospital stay, as well as impairment of postoperative liver regeneration have been reported.^{20,21} Numerous studies have shown a negative influence of SD on postoperative outcome,^{20,22,23} yet others could not reproduce this.²⁴⁻²⁹ This systematic review with

meta-analysis aimed to determine the influence of SD on short-term outcome after partial hepatectomy in patients with CRLM.

Methods

Criteria for considering studies for this review

An extensive protocol, which is available upon request, was written before the start of this study. This review was conducted and reported in compliance with the PRISMA and MOOSE guidelines, and followed the Cochrane protocol for prognostic factor reviews.³⁰⁻³² Studies were considered eligible for inclusion in this review when they met the following criteria: (I) studies comprising adults (≥ 18 years old) with CRLM, (II) who underwent minor or major partial hepatectomy, (III) with postoperative histological grading of SD in liver tissue distant from the tumour according to the scoring system of Rubbia-Brandt, (IV) and with registration of overall morbidity, liver-related morbidity, liver failure, or overall mortality (≤ 90 days or in-hospital) after liver resection. Case reports, comments/editorials, published abstracts, and reviews were rejected, in addition to records not covering the subject or including non-adults (age < 18 years). Cohort studies including patients who underwent liver surgery for malignancies other than CRLM were included albeit that this group comprised less than 30% of the total study population. No distinction was made between first and repeated resections, and studies with patients who underwent preoperative portal vein embolization (PVE) were allowed for inclusion since an effect of PVE on outcome in patients with SD was observed in a single study only.³³

Search methods for identification of studies

Search strategies in international databases Medline, Embase, Cochrane Central Register of Controlled Trials (CENTRAL), Latin American and Caribbean Health Sciences Literature (LILACS) and Cumulative Index to Nursing and Allied Health Literature (CINAHL) were performed between 01.01.2004 and 09.06.2015 using the following keywords (adjusted to the relevant database and including synonyms): “sinusoidal obstruction syndrome”, “hepatic veno-occlusive disease”, and “Stuart-Bras syndrome”. Publication date of the search strategy was set from January 2004 onwards because the widely used criterion for scoring SD from Rubbia-Brandt et al. was developed in this year.¹ The Embase strategy was independently peer reviewed by a second information specialist using the Canadian Agency for Drugs and Technologies in Health (CADTH) checklist.³⁴ No language

restrictions or other limitations were applied. Details of the search strategy can be found in Appendix 2.1 (supplementary data).

Data collection and analysis

Records were downloaded in EndNote® X7 and duplicates were automatically and manually removed. All abstracts were screened by two independent reviewers (KvM, JZ), and in the rare case of no consensus the abstract was considered for full-text scanning. One of the reviewers (KvM) screened citations of all full-text articles for additional records on the base of title or abstract. Previous research from the authors on this topic resulted in an extensive own library which was also checked for references. Records considered for full-text assessment were screened independently by two reviewers (KvM, JZ). Full-text articles were screened for inclusion in *qualitative* analysis and/or *quantitative* analysis. Articles without postoperative mortality in the study population were described qualitatively due to lack of contribution to quantitative analysis.

Data extraction and definitions

Data extraction was performed independently by two reviewers (KvM, JZ) using a data extraction form in Excel specifically created for this study. Any dissimilarity in data was discussed and solved by consensus. Inter-observer agreement was calculated with Cohen's kappa coefficient. All information on study design and characteristics, main outcomes and possible overlap in cohort data was recorded. SD was defined according to the grading system of Rubbia-Brandt et al.² In short; SD 0: absence of signs of SD; 1: mild SD (centrilobular involvement limited to one-third of the lobular area); 2: moderate SD (centrilobular involvement extending in two-thirds of the lobular area) and 3: severe SD (complete lobular involvement or centrilobular involvement extending to adjacent lobules with bridging congestion). In all analyses, 'SD' was defined as the presence of grade 2 or 3 SD whilst 'no SD' was defined as grade 0 or 1. We considered SD grade 2-3 clinically more relevant than grade 0-1, since rupture of sinusoidal wall integrity is present in grade 2 and higher. Major liver resections were defined as resection of ≥ 3 Couinaud segments. All outcomes were measured between 30 and 90 days after partial hepatectomy or during initial hospital admission ('in-hospital'). Primary outcomes were overall morbidity, liver failure, and overall mortality after liver resection. Secondary outcome was liver-related morbidity after partial hepatectomy. Overall morbidity was defined as any complication (i.e. surgical and medical, excluding death) after liver resection, irrespective of severity.³⁵ Mortality was defined as death due to any cause after liver resection. Since consensus on the definition of liver failure is lacking,³⁶⁻³⁸ definitions were specified in the results section. Liver-related morbidity was defined as

any liver-related complication (e.g. bile leakage, liver abscess, liver failure) occurring after partial hepatectomy, regardless of severity.³⁹

Quality assessment

Risk of bias of individual studies was determined using the Quality In Prognosis Studies (QUIPS) Risk of Bias Assessment Instrument for Prognostic Factor Studies.^{40,41} The QUIPS instrument is a checklist composed of the domains study participation, study attrition, prognostic factor measurement, outcome measurement, study confounding, and statistical analysis and reporting, and produces an estimation of the risk of bias with help of a rating (high, moderate or low risk) per domain. The following definitions were chosen by the authors for rating the overall risk of bias: 'overall low risk of bias' was ≤ 2 domains rated as moderate risk and the remaining domains as low, 'overall moderate risk of bias' was ≥ 3 domains rated as moderate risk and the remaining domains as low, 'overall high risk of bias' was ≥ 1 domain rated as high risk, independent of the rating of the remaining domains. The quality of evidence per primary or secondary outcome was evaluated using the modified Grading of Recommendations Assessment, Development and Evaluation (GRADE) framework for prognosis studies,^{30,42} which is composed of eight scoring fields and aims to give an objective scoring of quality of evidence per outcome specific for the hypothesis of the user. Its fixed scoring parameters and rationale for downgrading described in the Summary of Findings Table makes GRADE the most transparent and reproducible method currently available. The starting score is based on the study design and quality can be downgraded (and in specific cases upgraded) per field. Quality of evidence is defined as (I) high quality: the true effect lies close to the estimate of the effect, (II) moderate quality: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different, (III) low quality: the true effect may be substantially different from the estimate of the effect or (IV) very low quality: the true effect is likely to be substantially different from the estimate of effect.³⁰

Data handling and statistical methods

Meta-analyses of two or more studies per outcome were performed in Review Manager 5.3 and depicted in forest plots. The principal effect measures consisted of a pooled odds ratio (OR) with 95% confidence intervals (95% CI) calculated by the Mantel-Haenszel test for dichotomous outcomes, which was chosen because of low event rates and small sample sizes. A p-value < 0.05 was considered significant. A random effects model was chosen instead of fixed effects because the true effect size was expected to vary within different studies. Statistical heterogeneity was evaluated with the X^2 test, I^2 ,

and comparison of point estimates between studies and overlap of 95% confidence intervals. Clinical heterogeneity was investigated by comparing different patient populations (SD 2-3 vs. SD 0-1; SD 1-3 vs. SD 0) and different extents of liver resection (all resections vs. major resections only). In case of significant heterogeneity, defined as an $I^2 \geq 65\%$ and a X^2 test p-value of < 0.10 (considered significant due to a potentially low number of included studies in meta-analyses), the OR and 95% CI were omitted from the forest plot

Results

Search results

The conducted search resulted in a total of 2777 hits. Figure 2.1 shows the selection process of included studies in detail. After duplicate removal, 2007 hits remained. A total of 50 full-text articles were assessed for eligibility. Four more records were selected for full-text article scanning during reference checking, and another four potentially relevant records were selected from the own library. After application of the predefined inclusion and exclusion criteria on the total of 58 full-text articles by the two reviewers (KvM, JZ), 23 articles were included in *qualitative* synthesis. Inter-observer agreement kappa was 0.81 and dissimilarity was solved by discussion. Of the included studies for qualitative analysis, 13 studies contained data for *quantitative* analysis. Study and patient characteristics, main outcomes and main conclusions of the studies are summarized in Table 2.1.

Included studies

A total of eight studies could be included in quantitative analysis to estimate the effect of SD grade 2-3 vs. SD grade 0-1 on postoperative outcomes after partial hepatectomy. All studies but one were retrospective cohort studies which enrolled at least 50 patients.⁵⁶ Each study was published in an international, peer-reviewed journal between 2006 and 2013. Postoperative morbidity could be evaluated in two studies comprising a total of 248 patients,^{26,44} and in two studies encompassing a total of 319 patients, postoperative liver failure was studied.^{47,56} Three studies investigated postoperative mortality in a total of 702 patients,^{21,55,56} and postoperative liver-related morbidity was evaluated in two studies with a total of 147 patients.^{22,26} For each of above outcomes, a single study addressed the effect of SD in patients undergoing major liver resection.^{26,53}

Figure 2.1 Selection process of included studies. This flowchart shows the exact steps that were taken from the search strategy until final inclusion of articles in qualitative and quantitative analysis, respectively. SD, sinusoidal dilatation; CINAHL, Cumulative Index to Nursing and Allied Health Literature; CENTRAL, Cochrane Central Register of Controlled Trials; LILACS, Latin American and Caribbean Health Sciences Literature; CRLM, colorectal liver metastases

Table 2.1 Summary of study characteristics and risk of bias assessment.
This table contains study and patient characteristics, main outcomes and main conclusions of the included studies.

Name	Year	Country	n	Comparison	Study design	Quant/ Qual	QUIPS score	Main outcome
Aloysius ⁴³	2007	UK	50	3 vs. 2 vs. 1 vs. 0	RCohS	Qual	High	Folfox-4 therapy is associated with SD and hepatic steatosis
Brouquet ⁴⁴	2009	France	146	2-3 vs. 0-1	RCohS	Quan	High	Morbidity rate is significantly increased in patients with CALI compared to patients without CALI
Gomez-Ramirez ²²	2010	Spain	45	2-3 vs. 0-1	PCohS	Quan	Low	Patients treated with oxaliplatin have a higher incidence of SOS, increase in liver complications and longer mean hospital stay
Hubert ⁴⁵	2010	Belgium	114	3 vs. 0-2	RCohS	Qual	Low	Neoadjuvant Ctx is significantly associated with SD but does not affect postoperative clinical outcome
Imai ¹⁹	2014	Japan	55	2-3 vs. 0-1	RCohS	Qual	High	Ox-based Ctx is related to SD, and patients with SD have a significantly higher increase in splenic volume
Kandutsch ²⁴	2008	Austria	63	3 vs. 2 vs. 1 vs. 0	RCohS	Qual	High	Sinusoidal dilatation is not associated with postoperative morbidity
Karoui ⁴⁶	2006	France	67	1-3 vs. 0	RCohS	Qual	Low	Prolonged neoadjuvant Ctx alters liver parenchyma and increases morbidity after major resection under total hepatic vascular exclusion
Kishi ⁴⁷	2010	Italy	219	2-3 vs. 0-1	RCohS	Quan	Low	SI is not a risk factor for the development of liver insufficiency
Komori ²⁵	2010	Japan	27	3 vs. 2 vs. 1 vs. 0	RCohS	Qual	High	Folfox-4 therapy is associated with SD. Ctx does not increase postoperative morbidity and mortality in patients
Makowiec ²⁶	2011	Germany	102	2-3 vs. 0-1	RCohS	Quan	Low	Neither preoperative Ctx nor liver injury increase perioperative morbidity
Mentha ¹⁵	2009	Switzerland	23	3 vs. 2 vs. 1 vs. 0	RCohS	Qual	High	Neoadjuvant Ctx followed by two-step hepatectomy with right portal vein occlusion is feasible and safe
Miura ⁴⁸	2011	Japan	14	2-3 vs. 0-1	RCohS	Qual	High	APRI and SVI help indicate the risk of CALI. The incidence of SD was significantly higher in the SVI $\geq 30\%$ compared to the SVI $< 30\%$ group
Nakano ²⁰	2008	France	90	1-3 vs. 0	RCohS	Quan	Mod	SI is significantly associated with higher morbidity and longer hospital stay in patients undergoing a major hepatectomy
^a Narita ³³	2011	France	42/45	1-3 vs. 0	RCohS	Quan	Low	SOS has a negative impact on hypertrophy following PVE and is associated with a higher risk of postoperative liver failure after major hepatectomy

Table 2.1 (continued)

Name	Year	Country	n	Comparison	Study design	Quant/ Qual	QUIPS score	Main outcome
^a Narita ⁴⁹	2012	France/ USA	101	1-3 vs. 0	RCohS	Quan	Low	Ox-based Ctx is associated with a significantly increased occurrence of SD, but SD does not impair early postoperative outcome
Nguyen-Khac ⁵⁰	2013	France	50	1-3 vs. 0	RCohS	Quan	Low	Ctx of CRLM induces SD related to oxaliplatin, without impact on postoperative mortality
^b Pessaux ⁵¹	2010	France	52	1 vs. 2	RCohS	Qual	High	The addition of cetu to neoadjuvant Ctx does not increase postoperative morbidity or the occurrence of SD
^b Pessaux ⁵²	2010	France	72	1 vs. 2	RCohS	Qual	High	The addition of cetu or bev to neoadjuvant Ctx does not increase postoperative morbidity or the occurrence of SD
Soubrane ⁵³	2010	France	78	2-3 vs. 0-1	RCohS	Quan	Low	High grade SI leads to an increased risk of postoperative complications following major resection
Takamoto ²³	2010	Japan	51	3 vs. 2 vs. 1 vs. 0	RCohS	Qual	Mod	The amount of blood loss during surgery is significantly higher in patients with liver injury
Tamandl ²¹	2011	Austria	196	2-3 vs. 0-1	RCohS	Quan	High	SD due to ox-based Ctx can lead to early recurrence and decreased survival
Van der Pool ⁵⁴	2012	Netherlands	104	1-3 vs. 0	RCohS	Quan	Low	Neither duration of ox-based Ctx nor time interval between cessation of ox-based Ctx and surgery are associated with postoperative complications
Vauthey ⁵⁵	2006	USA/Italy	406	2-3 vs. 0-1	RCohS	Quan	Low	Preoperative ox-based Ctx is associated with SI but not with increased morbidity or mortality rates
Vigano ⁵⁶	2012	Italy	100	2-3 vs. 0-1	PCohS	Quan	Low	The rate of liver dysfunction is high among patients with moderate to severe CALIs
Wolf ⁵⁷	2013	USA	384	1-3 vs. 0	RCohS	Quan	Low	No association is found between oxaliplatin use and SI or SI and postoperative morbidity

RCohS, retrospective cohort study; PCohS, prospective cohort study; Quan, quantitative and qualitative data; Qual, solely qualitative data; CALI, chemotherapy-associated liver injury; Ctx, chemotherapy; APRI, aspartate transaminase to platelet ratio index; ox-based, oxaliplatin-based; cetu, cetuximab; bev, bevacizumab; SVI, splenic volume increase; SOS, sinusoidal obstruction syndrome; SD, sinusoidal dilatation; SI, sinusoidal injury; mod, moderate; a Considerable overlap between patient cohorts was confirmed by the authors, larger sample size and broader inclusion criteria contributed to the decision to solely include Narita (2012) in quantitative analysis; b Considerable overlap between patient cohorts was confirmed by the authors, larger sample size and broader inclusion criteria contributed to the decision to solely include Pessaux (2010, n=72) in qualitative analysis.

Patient characteristics

All cohort studies included patients who underwent minor or major hepatectomy and of whom liver quality was assessed postoperatively according to the grading system of Rubbia-Brandt.¹ In total 63% of patients who underwent surgery were male, and median age was 61 years (range: 18 to 89). CRLM was the indication for liver surgery in all patients.

Primary outcomes

Postoperative morbidity

A meta-analysis on the influence of SD grade 2-3 vs. SD grade 0-1 on postoperative morbidity was conducted and depicted in Figure 2.2A. The overall effect of SD vs. no SD on postoperative morbidity showed a p-value of 0.40 with an OR of 1.26 (95% CI 0.74-2.15). Tests evaluating consistency of results showed an I^2 of 0% and a X^2 test p-value of 0.74. Although the use of random effects was deliberately chosen, testing with fixed effects did not have influence on the results (OR 1.26, 95% CI 0.73-2.15, $p=0.40$). SD was no risk factor for morbidity in studies where original data was not provided for quantitative analysis.^{21,24,50,55}

In patients undergoing major hepatectomy ($n=59$, hemihepatectomy or extended hemihepatectomy), 17 out of 29 patients (59%) with SD developed morbidity vs. 19 out of 30 patients (63%) in patients without SD (not depicted in forest plot).²⁶

Figure 2.2a Postoperative overall morbidity.

Meta-analysis on the influence of sinusoidal dilatation (SD) grade 2-3 versus SD grade 0-1 on the morbidity rate due to any cause after partial hepatectomy for colorectal liver metastases

Postoperative liver failure

The effect of SD on postoperative liver failure (PLF) could be tested in two studies (Figure 2.2B). In the study of Kishi et al., liver failure was defined as peak total bilirubin value $>120 \mu\text{mol/L}$ in the postoperative course,⁴⁷ while Vigano et al. applied serum

bilirubin >50 µmol/L and/or prothrombin time <50% on or after postoperative day (POD) 5.⁵⁶ An OR of 1.03 (95% CI 0.15-6.89) was found with a p-value of 0.98 for SD. Significant heterogeneity between studies was reflected in an I^2 of 65% and p-value of X^2 test of 0.09, and overlap in 95% CI was barely present. Totals were subsequently omitted from the forest plot. One qualitative study showed no PLF in both the patients with or without SD.²³ The study of Soubrane et al. was the only study that looked at postoperative liver failure in major resections only, and defined liver failure as a prothrombin time <50% and serum bilirubin >50 µmol/L on POD5.⁵³ Liver failure occurred in eight of 38 patients with SD vs. zero in 13 patients without SD (not depicted in forest plot).

Figure 2.2b Postoperative liver failure.
Meta-analysis on the influence of sinusoidal dilatation (SD) grade 2-3 versus SD grade 0-1 on the liver failure rate after partial hepatectomy for colorectal liver metastases.

Postoperative mortality

Three studies were available for examining the influence of SD on postoperative mortality (Figure 2.2C), with a total of 16 events. The test for overall effect showed a p-value of 0.82 with an OR of 1.21 (95% CI 0.23-6.35). Statistical testing for heterogeneity showed an I^2 of 0% and p-value of 0.79. Assumption of fixed effects barely influenced the results (OR 1.15, 95% CI 0.24-5.51, p-value=0.86). Multiple studies described zero mortality in groups with or without SD,^{15,19,22-25,43,48,52} or no increase in mortality rate in patients with SD⁴⁷.

Figure 2.2c Postoperative overall mortality.
Meta-analysis on the influence of sinusoidal dilatation (SD) grade 2-3 versus SD grade 0-1 on the mortality rate due to any cause after partial hepatectomy for colorectal liver metastases.

As described before, Soubrane et al. presented a subgroup analysis in patients who underwent major liver resection.⁵³ In this study, two postoperative deaths occurred within 90 days in the SD group that consisted of 38 patients (4%). Thirteen patients did not have SD with zero mortality.

Secondary outcome

Liver-related morbidity

Two studies were available for estimating the influence of SD on postoperative liver-related morbidity (Figure 2.2D). The study of Gomez-Ramirez et al. included the following complications: biliary fistula, haemorrhage, abscesses, uninfected collections and liver failure,²² while Makowiec et al. included hepatic insufficiency, bilioma and/or symptomatic ascites requiring interventional or medical treatment.²⁶ An OR of 3.52 (95% CI 0.31-39.91) was found with a p-value of 0.31 for SD. I^2 was 83% with a p-value of X^2 test of 0.01. Overlapping of intervals between included studies was barely present; hence totals were omitted from the forest plot. In major hepatectomy only, eight out of 30 patients without SD vs. 10 out of 29 patients with SD developed liver-related complications.²⁶

Figure 2.2d Postoperative liver-related morbidity.

Meta-analysis on the influence of sinusoidal dilatation (SD) grade 2-3 versus SD grade 0-1 on the liver-related morbidity rate after partial hepatectomy for colorectal liver metastases.

Sub group analysis

Five studies compared SD grade 1-3 with SD grade 0. Analyses on these subgroups were performed and resulting forest plots are presented in Appendix 2.2. In summary, the effect of SD grade 1-3 on the primary outcome postoperative morbidity could be tested in three studies ($n=577$),^{20,54,57} liver failure in one study ($n=90$),²⁰ and mortality in two studies ($n=433$).^{50,57} Again, the risk for postoperative overall morbidity after minor or major liver resection in the SD group tended to be higher (OR 1.53, 95% CI 0.96-2.44, $p=0.08$) than in the group without SD (Appendix 2.2A). There was limited evidence of effect (one study) of SD on PLF, and therefore no meta-analysis was conducted on this

outcome. This study showed a higher incidence of PLF, which was defined as a serum bilirubin $>50 \mu\text{mol/L}$ and a prothrombin time $<50\%$ on POD5 or thereafter, in the SD group (three out of 38 patients with SD vs. zero out of 52 patients without SD).²⁰ Estimate points of effect for postoperative mortality resulted in an OR of 0.51 and p-value of 0.54 (Appendix 2.2B). Statistical heterogeneity analysis revealed $I^2=0\%$ and p-values of 0.77 and 0.40 for postoperative overall morbidity and mortality, respectively. No mortality was observed in patient groups with and without SD in two studies.^{20,54} Secondary outcome measure liver-related morbidity could be tested in two studies^{20,57} and yielded an OR of 2.22 (95% CI 0.34-14.32, $p=0.40$) and an I^2 of 64% ($p=0.10$) (Appendix 2.2C).

For major resections only, forest plots were constructed for postoperative overall morbidity and liver failure (Appendix 2.3). Two studies that included a total of 137 patients were available for a meta-analysis on postoperative morbidity (Appendix 2.3A).^{20,49} An OR of 2.00 (95% CI 0.12-33.34) with a p-value of 0.63 for SD were found. I^2 was 83% with a p-value of X^2 test of 0.02, and totals were omitted from the forest plot. The same two studies were available for a meta-analysis on postoperative liver failure (Appendix 2.3B). Whereas Nakano et al. defined postoperative liver failure as a serum bilirubin $>50 \mu\text{mol/L}$ and a prothrombin time $<50\%$ on or after POD5,²⁰ Narita et al. defined this as a serum bilirubin $>50 \mu\text{mol/L}$ and a prothrombin time $<50\%$ on POD5 and/or a postoperative serum bilirubin $>120 \mu\text{mol/L}$.⁴⁹ An OR of 2.67 (95% CI 0.94-7.53) with a near-significant p-value of 0.06 for SD was found. No heterogeneity was detected as reflected in an I^2 of 0% with a X^2 test p-value of 0.73. No mortality in patients with or without SD was observed in two studies on major hepatectomy.^{20,46} Liver-related morbidity was seen in the study of Nakano et al.,²⁰ with three patients showing liver-related morbidity in the patient group with SD ($n=20$) versus one patient in the patient group without SD ($n=16$).

Quality of the included studies

Risk of bias assessment

Risk of bias was assessed using the modified QUIPS checklist^{40,41} and overall ratings are depicted in Table 2.1. Thirteen articles showed an overall low risk of bias, two studies showed moderate risk of bias and ten articles were appraised as having a high risk of bias. The assessment of risk of bias per domain and sub domain for individual studies can be found in Appendix 2.4.

GRADE assessment

Study findings were assessed with the modified GRADE checklist^{42,58} and are shown in the Summary of Findings Table (Table 2.2). Footnotes provide detailed information about the rationale for downgrading. In short, evidence for all outcomes was rated as *very low*. Each outcome was downgraded on the base of study design (phase 1 explanatory studies). Other reasons for downgrading were indirectness in definition or time period of outcome measures and imprecision in data as shown by forest plots. Only QUIPS domain 1 to 4 were taken into account for downgrading, since domains 5 and 6 assess quality of the statistical analysis which did not influence quality of the current meta-analysis because we extracted only raw data from the manuscripts. A detailed rating per domain can be found in Appendix 2.5. A Summary of Findings Table for outcomes on SD grade 1-3 vs. 0 and a detailed rating per domain can be found in Appendix 2.6 and 2.7.

Discussion

The present study found no significant influence of moderate to severe SD (grade 2-3) in comparison to no or mild SD (grade 0-1) on outcome after partial hepatectomy. Likewise, no influence on postoperative outcome was apparent when comparing mild to severe SD (grade 1-3) to no SD (grade 0). After data extraction, all studies were subjected to assessment with the QUIPS and GRADE tools to grade the quality of included evidence, strength of recommendations and risk of bias. Critical evaluation subsequently showed a low to high risk of bias for individual studies and very low quality of outcome-specific evidence, thereby leading to limited confidence in the provided evidence with regards to our hypothesis. An important explanation for less trust in the provided evidence is the study design of included articles. All but one study consisted of explanatory phase 1 studies, which are performed in early phases of investigation to generate a hypothesis and are considered weaker evidence than studies which confirm the independent effect of a specific prognostic factor on outcome (phase 2 studies), or studies that explore the underlying mechanism for prognosis of certain diseases (phase 3 studies).^{30,59} Moreover, variation in inclusion criteria, sample size differences, wide confidence intervals crossing the null value, and different definitions of outcomes, were factors that negatively affected the quality of the evidence specified for our hypothesis. In light of these findings, no solid conclusions can be drawn and this study cannot provide clinical advice on the topic.

Table 2.2 Modified grade summary of findings table for the influence of SD grade 2-3 vs. SD grade 0-1 on outcome after partial hepatectomy.
A table depicting the influence of sinusoidal dilatation (SD) grade 2-3 versus SD grade 0-1 on morbidity, liver failure, mortality, and liver-related morbidity after partial liver resection. The grading of recommendations assessment, development and evaluation tool (GRADE) is applied per outcome and a subsequent rationale for the overall GRADE assessment score is given.

Patient or population: patients with CRLM Setting: hospital Prognostic factor: SD grade 2-3					
Outcomes	Number of participants	Number of studies	Estimated effect size (95% CI)	GRADE assessment [†]	
Morbidity	248	2	OR 1.26 (0.74 to 2.15)	Very low ^{b,c,f}	
Follow-up: 30 to 90 days or in-hospital					
Liver failure	319	2	Omitted due to significant heterogeneity	Very low ^{b,c,d,e,f}	
Follow-up: 30 to 90 days or in-hospital					
Mortality	702	3	OR 1.21 (0.23 to 6.35)	Very low ^{e,f}	
Follow-up: 30 to 90 days or in-hospital					
Liver-related morbidity	147	2	Omitted due to significant heterogeneity	Very low ^{b,c,d,e}	
Follow-up: 30 to 90 days or in-hospital					

^a In the exemplar review for prognostic studies of Hayden et al.³⁰, phase 2 and 3 explanatory studies start with a high grade score (four points), whereas phase 1 explanatory studies start with a moderate score (three points). Since all outcomes consisted for ≥50% of phase 1 studies, the starting score for all outcomes was set on three points;^b The definition of the outcome was not clearly stated in all included articles or there were differences in outcome definition between the included studies for this outcome;^c The time period for measurement of the outcome was not clearly stated in ≥50% of the included studies;^d (Almost) no overlap in 95% confidence intervals and estimate points of effect could be found on both sides of the null line in meta-analysis for this outcome;^e There was significant unexplained heterogeneity for this outcome as defined by an I²≥65% and a p-value <0.10; ^f This outcome contains imprecise results, defined as an inclusion of only two studies and/or an insufficient sample size, wide confidence intervals or confidence intervals crossing the null value in ≥50% of studies.

GRADE, Grading of Recommendations Assessment, Development and Evaluation; SD, sinusoidal dilatation; CRLM, colorectal liver metastases; CI, confidence interval; OR, odds ratio.

Some studies showed contrasting data, which may be partially explained by different inclusion criteria. While patients who received bevacizumab were intentionally excluded in some studies,^{26,50} other studies did allow inclusion of these patients^{22,47}. Bevacizumab, an angiogenesis inhibitor that inhibits tumour growth by binding to vascular endothelial growth factor, is often co-administered in oxaliplatin-based chemotherapy regimens for patients with CRLM.⁶⁰ Apart from the ongoing discussion about the potential benefit of prevention or reversibility of histological injury,^{2,61} bevacizumab itself may induce relevant toxic side effects which can affect recovery after partial liver resection.⁶²⁻⁶⁴ Moreover, it is usually advised to end chemotherapy, particularly when bevacizumab is co-administered, at least five weeks before liver surgery.⁶⁵ Studies included in this systematic review report a range from 2 to 9 weeks for the interval between the last cycle of chemotherapy and surgery. It is currently unclear whether pathological characteristics of SOS are reversible, if a lower grade of SOS is linked to a higher likeliness of reversibility, and in which time frame this would occur. Patients with a long time interval between chemotherapy cessation and surgery were observed to have less SD compared to a short time interval,²⁰ whereas irreversibility of SOS and even deterioration in time have been described by Mentha et al.¹⁵

Likewise, a link between the number of administered cycles of chemotherapy and grade of liver injury is uncertain, and the median number of administered cycles in this meta-analysis ranged from 6 to 12. Whereas Karoui et al. found that the morbidity rate was correlated with the number of chemotherapy cycles,⁴⁶ this correlation was not confirmed by Van Der Pool et al. who compared patients who received less than six cycles with those who received six or more cycles.⁵⁴ One could speculate that patients with longer duration of chemotherapy may have had more extensive disease and therefore more complex surgical interventions. Almost all studies corrected for the extent of liver resection, but extrahepatic procedures and vascular reconstructions were not taken into account in more than half of studies. The impact of these procedures on outcome can be substantial.^{66,67}

In patients with a critical future liver remnant due to anticipated extensive surgery, portal vein embolization (PVE) is the preferred procedure to induce pre-operative enlargement of the future liver remnant.⁶⁸ The effect of SD on liver regeneration after PVE has been investigated in one study, in which PVE had a negative effect on postoperative liver regeneration.³³ Moreover, post-PVE histopathological changes in a previous report were the rationale for Vauthey et al.⁵⁵ and Brouquet et al.⁴⁴ to exclude these patients from their cohorts.⁶⁹ This resulted in a less complete reflection of the general surgical population. However, inclusion of these patients may have an impact on

postoperative outcomes either by a diminished hypertrophic response or because this group of patients often undergoes extended hepatectomies and is already at higher risk.

With regard to variations in definitions, outcome after liver resection was expressed in numerous terms and time frames. Mortality was assessed within 30, 60, or 90 days after surgery or during hospital admission, and morbidity was described as overall, medical, surgical, liver-related or infectious, with no consensus on employed definitions. This underscores the necessity of a uniform outcome set for liver surgery in order to ensure clear and consistent clinically relevant data, and to allow comparisons between future trials and cohort studies in meta-analyses.³⁹

On the basis of stated differences in inclusion criteria and outcome, which all might have influenced the outcome after liver resection to an unknown extent, ideally a phase two study in which all confounders are corrected for in a statistically appropriate way should be conducted. Our group recently initiated such a study in the form of an individual patient data meta-analysis.⁷⁰

Conclusion

This study aimed to evaluate the influence of moderate to severe SD on outcome in patients undergoing partial hepatectomy for CRLM. Although many individual studies suggest a negative impact on postoperative (liver-related) morbidity, liver failure and mortality, the present meta-analysis could not confirm this data. However, trust in the obtained evidence was low and therefore no solid conclusions can be drawn. This study emphasizes the importance of critical risk of bias assessment and evaluation of quality in meta-analyses, to provide the most robust level of evidence for clinical decision making. It also highlights the need for unambiguous definitions of outcome in surgical oncology.

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Appendix 2.1. Full electronic search

The full electronic search strategy that was performed for the present study in multiple international databases

Searches were limited to retrieve results from 01.01.2004-09.06.2015

- Medline (OvidSP): 2004-2015/05/WK5
- Medline In-Process Citations & Daily Update (OvidSP): up to 2015/06/08
- Embase (OvidSP): 2004-2015/06/08
- Cochrane Central Register of Controlled Trials (CENTRAL) (Wiley) 2004-Issue 5, 2015/05
- LILACS (Latin American and Caribbean Health Sciences Literature) (Internet) <http://lilacs.bvs.alud.org/en/>: 2004-2015/06/03
- CINAHL (Cumulative Index to Nursing and Allied Health Literature) (EBSCO): 2004-2015/06/05

Strategies

Medline (OvidSP): 2004-2015/05/WK5

Searched 9.6.15

- 1 Hepatic Veno-Occlusive Disease/ (1105)
- 2 ((venocclusi\$ or venoocclusi\$ or veno occlusi\$) adj2 (liver or livers or hepato\$ or hepatic\$)).ti,ab,ot. (667)
- 3 Stuart bras syndrome.ti,ab,ot. (3)
- 4 ((SOS or VOD or HVOD) adj5 (liver or livers or hepatic\$ or hepato\$)).ti,ab,ot. (460)
- 5 (sinusoid\$ adj2 (obstruct\$ or dilat\$ or injur\$ or damage\$)).ti,ab,ot. (898)
- 6 or/1-5 (2164)
- 7 exp animals/ not (exp animals/ and humans/) (4056152)
- 8 6 not 7 (1785)
- 9 limit 8 to yr="2004 -Current" (742)

Medline In-Process Citations (OvidSP): up to 2015/06/08

Medline Daily Update (OvidSP): up to 2015/06/08

Searched 9.6.15

- 1 Hepatic Veno-Occlusive Disease/ (0)
- 2 ((venocclusi\$ or venoocclusi\$ or veno occlusi\$) adj2 (liver or livers or hepato\$ or hepatic\$)).ti,ab,ot. (26)
- 3 Stuart bras syndrome.ti,ab,ot. (0)
- 4 ((SOS or VOD or HVD) adj5 (liver or livers or hepatic\$ or hepato\$)).ti,ab,ot. (22)
- 5 (sinusoid\$ adj2 (obstruct\$ or dilat\$ or injur\$ or damage\$)).ti,ab,ot. (91)
- 6 or/1-5 (112)
- 7 exp animals/ not (exp animals/ and humans/) (2153)
- 8 6 not 7 (112)
- 9 limit 8 to yr="2004 -Current" (110)

Embase (OvidSP): 2004-2015/6/8

Searched 9.6.15

- 1 liver venoocclusive disease/ (1279)
- 2 ((venocclusi\$ or venoocclusi\$ or veno occlusi\$) adj2 (liver or livers or hepato\$ or hepatic\$)).ti,ab,ot. (887)
- 3 Stuart bras syndrome.ti,ab,ot. (3)
- 4 ((SOS or VOD or HVD) adj5 (liver or livers or hepatic\$ or hepato\$)).ti,ab,ot. (769)
- 5 (sinusoid\$ adj2 (obstruct\$ or dilat\$ or injur\$ or damage\$)).ti,ab,ot. (1514)
- 6 or/1-5 (3329)
- 7 animal/ (1665207)
- 8 animal experiment/ (1854326)
- 9 (rat or rats or mouse or mice or murine or rodent or rodents or hamster or hamsters or pig or pigs or porcine or rabbit or rabbits or animal or animals or dogs or dog or cats or cow or bovine or sheep or ovine or monkey or monkeys).ti,ab,ot,hw. (5941840)
- 10 or/7-9 (5941840)
- 11 exp human/ (16002764)
- 12 human experiment/ (337714)
- 13 or/11-12 (16004207)
- 14 10 not (10 and 13) (4709608)
- 15 6 not 14 (2805)
- 16 limit 15 to yr="2004 -Current" (1833)

Cochrane Central Register of Controlled Trials (CENTRAL) (Wiley): Issue 5/ May 2015

Searched 11.3.15

- #1 MeSH descriptor: [Hepatic Veno-Occlusive Disease] explode all trees 40
- #2 ((venocclusi* or venoocclusi* or veno occlusi*) near/2 (liver or livers or hepato* or hepatic*)):ti,ab,kw 62
- #3 Stuart bras syndrome:ti,ab 1
- #4 ((SOS or VOD or HVOD) near/5 (liver or livers or hepatic* or hepato*)):ti,ab,kw 36
- #5 (sinusoid* near/2 (obstruct* or dilat* or injur* or damage*)) .ti,ab,ot. 0
- #6 #1 or #2 or #3 or #4 or #5 Publication Year from 2004 to 2015 40

CENTRAL results = 30

Literature in the Health Sciences in Latin America and the Caribbean (LILACS) (Internet): 2004-2015/06/03

Searched 9.6.15

Advanced search

Limited to 2004-2015

Limited to Humans only

Limited to: LILACS

(tw:((mh: c06.552.360 OR mh: c14.907.460 OR mh: hepatic veno-occlusive disease OR sinusoidal obstruction OR sinusoidal injury OR "stuart bras syndrome" OR "Enfermedad Veno-Oclusiva Hepática" OR "Hepatopatía Veno-Oclusiva")) OR (tw:(((sos OR vod OR venocclusi* OR venoocclusi* OR veno-occlusi*) AND (liver OR livers OR hepatic* OR hepato*)))) OR (tw:((sinusoid* AND (obstruct* OR dilat* OR injur* OR damage*))))

N=26

CINAHL (Cumulative Index to Nursing and Allied Health Literature) (EBSCO): 2004-2015/06/05

Searched 9.6.15

- S1 MH Hepatic Veno-Occlusive Disease Search modes 0
- S2 TI ((venocclusi* or venoocclusi* or veno occlusi*) N2 (liver or livers or hepato* or hepatic*)) 7
- S3 AB ((venocclusi* or venoocclusi* or veno occlusi*) N2 (liver or livers or hepato* or hepatic*)) 13
- S4 TI Stuart bras syndrome 0
- S5 AB Stuart bras syndrome 0
- S6 TI ((SOS or VOD or HVOD) N5 (liver or livers or hepatic* or hepato*)) 0

S7 AB ((SOS or VOD or HVOD) N5 (liver or livers or hepatic* or hepato*)) 6
S8 TI (sinusoid* N2 (obstruct* or dilat* or injur* or damage*)) 14
S9 AB (sinusoid* N2 (obstruct* or dilat* or injur* or damage*)) 19
S10 S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7 OR S8 OR S9 44
S11 S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7 OR S8 OR S9 Limiters - Published Date:
20040101-20151231 36

Appendix 2.2. Sub analyses of postoperative short-term outcomes SD grade 1-3 vs. SD grade 0

Meta-analyses on the influence of sinusoidal dilatation (SD) grade 1-3 versus SD grade 0 on the complication rate after partial hepatectomy for colorectal liver metastases

Appendix 2.2a. Postoperative overall morbidity

Meta-analysis on the influence of sinusoidal dilatation (SD) grade 1-3 versus SD grade 0 on the morbidity rate due to any cause after partial hepatectomy for colorectal liver metastases

Appendix 2b. Postoperative overall mortality

Meta-analysis on the influence of sinusoidal dilatation (SD) grade 1-3 versus SD grade 0 on the mortality rate due to any cause after partial hepatectomy for colorectal liver metastases

Appendix 2.2c. Postoperative liver-related morbidity

Meta-analysis on the influence of sinusoidal dilatation (SD) grade 1-3 versus SD grade 0 on the liver-related morbidity rate after partial hepatectomy for colorectal liver metastases

SD, sinusoidal dilatation; M-H, Mantel-Haenszel; Random, random effects model; 95% CI, 95% confidence interval

Appendix 2.3. Sub analyses of postoperative short-term outcomes SD grade 1-3 vs. SD grade 0 – major liver resections

Meta-analyses on the influence of sinusoidal dilatation (SD) grade 1-3 versus SD grade 0 on the complication rate after major hepatectomy for colorectal liver metastases

Appendix 2.3a. Postoperative overall morbidity

Meta-analysis on the influence of sinusoidal dilatation (SD) grade 1-3 versus SD grade 0 on the morbidity rate due to any cause after major hepatectomy for colorectal liver metastases

Appendix 2.3b. Postoperative liver failure

Meta-analysis on the influence of sinusoidal dilatation (SD) grade 1-3 versus SD grade 0 on the liver failure rate after major hepatectomy for colorectal liver metastases

SD, sinusoidal dilatation; M-H, Mantel-Haenszel; Random, random effects model; 95% CI, 95% confidence interval

Appendix 2.4. Quality in prognostic studies tool (quips) detailed risk of bias assessment of all individual included studies

A detailed risk of bias assessment using the quality in prognostic studies tool (QUIPS) checklist. Per study, every criterion of the six domains is rated and subsequently a summary score per domain is assigned. Eventually, a total score is calculated

[illegible]

	Aloysius ⁴³	Brouquet ⁴⁴	Gómez-Ramírez ²²	Hubert ⁴⁵	Imai ¹⁹	Kandutsch ²⁴	Karoui ⁴⁶	Kishi ⁴⁷	Komori ²⁵	Makowiec ²⁶	Mentha ¹⁵	Miura ⁴⁸	Nakano ²⁰	Narita ⁴⁹	Nguyen-Khac ⁵⁰	Pessaux ⁵²	Soubrane ⁵³	Takamoto ²³	Tamandi ²¹	Van der Pool ⁵⁴	Vauthey ⁵⁵	Vigano ⁵⁶	Wolf ⁵⁷
3. Prognostic Factor Measurement																							
Definition of the PF	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Valid and Reliable Measurement of PF	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Reporting continuous variables	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Method and Setting of PF Measurement	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Proportion of data on PF available for analysis	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Method used for missing data	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
PF Measurement Summary	low	low	low	low	low	low	low	low	low	low	high	low	low	low	low	low	low	low	low	low	low	low	low
4. Outcome Measurement																							
Definition of the Outcome	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Valid and Reliable Measurement of Outcome	✓	?	✓	✓	✓	✓	✓	✓	✓	✓	✓	?	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Method and Setting of Outcome Measurement	✓	?	✓	✓	✓	✓	✓	✓	✓	✓	✓	?	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Outcome Measurement Summary	mod	high	low	low	mod	mod	low	mod	mod	mod	mod	high	mod	mod	low	low	low	low	mod	mod	mod	low	mod

	Aloysius ⁴³	Brouquet ⁴⁴	Gómez-Ramírez ²²	Hubert ⁴⁵	Imai ¹⁹	Kandutsch ²⁴	Karoui ⁴⁶	Kishi ⁴⁷	Komori ²⁵	Makowiec ²⁶	Mentha ¹⁵	Miura ⁴⁸	Nakano ²⁰	Narita ⁴⁹	Nguyen-Khac ⁵⁰	Pessaux ⁵²	Soubrane ⁵³	Takamoto ²³	Tamandl ²¹	Van der Pool ⁵⁴	Vauthey ⁵⁵	Vigano ⁵⁶	Wolf ⁵⁷
5. Study Confounding																							
Important Confounders Measured	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Definition of the confounding factor	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Valid and Reliable Measurement of Confounders	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Method and Setting of Confounding Measurement	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Method used for missing data	✓	✓	✓	✓	✓	✓	✓	?	✓	✓	?	✓	✓	✓	✓	✓	✓	✓	?	✓	✓	✓	?
Appropriate Accounting for Confounding in study design	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
Appropriate Accounting for Confounding in analysis	×	×	×	×	×	✓	×	✓	×	×	×	×	×	✓	×	×	×	×	×	×	×	×	×
Study Confounding Summary	mod	mod	mod	mod	mod	low	mod	mod	mod	mod	high	mod	mod	low	mod	mod	mod	mod	high	mod	mod	mod	mod

	Aloysius ⁴³	Brouquet ⁴⁴	Gómez-Ramírez ²²	Hubert ⁴⁵	Imai ¹⁹	Kandutsch ²⁴	Karoui ⁴⁶	Kishi ⁴⁷	Komori ²⁵	Makowiec ²⁶	Mentha ¹⁵	Miura ⁴⁸	Nakano ²⁰	Narita ⁴⁹	Nguyen-Khac ⁵⁰	Pessaux ⁵²	Soubrane ⁵³	Takamoto ²³	Tamandi ²¹	Van der Pool ⁵⁴	Vauthey ⁵⁵	Vigano ⁵⁶	Wolf ⁵⁷
6. Statistical Analysis and Reporting																							
Presentation of analytical strategy	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Appropriate model building	✗	✗	✓	✓	✗	✓	✓	✓	✗	✓	✗	✗	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓
Adequate statistical model	✗	✗	✓	✓	✗	✓	✓	✓	✗	✓	✗	✗	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓
Reporting of results	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Statistical Analysis and Presentation Summary	high	high	mod	low	high	low	mod	low	high	low	high	high	mod	low	low	low	high	low	mod	low	low	low	mod
Overall assessment	high	high	low	low	high	high	low	low	high	low	high	high	mod	low	low	low	high	low	mod	high	low	low	low

PF, prognostic factor; ✓, yes; ✓-, partial; ✗, no; ?, unsure; mod, moderate; n/a, not applicable.

Appendix 2.5. Detailed quality assessment of all outcomes with the grading of recommendations assessment, development and evaluation tool (grade)

The influence of sinusoidal dilatation (SD) grade 2-3 compared to SD grade 0-1 on morbidity, liver failure, mortality, and liver-related morbidity after partial hepatectomy is depicted. Per outcome, specific domains of the grading of recommendations assessment, development and evaluation tool (GRADE) are scored and a subsequent overall score is calculated

The influence of SD grade 2-3 compared to SD grade 0-1 on short-term outcome after partial hepatectomy												
Patient or population: patients with CRLM Setting: hospital Prognostic factor: SD grade 2-3				GRADE factors								
Outcomes	No of participants (studies)	Estimated effect size (95% CI)	Phase	Study limitations	Inconsistency	Indirectness	Imprecision	Publication bias	Moderate/large effect size	Dose effect	Overall score	
Morbidity	248 (2)	OR 1.26 (0.74 to 2.15)	1	✗	✓	✗	✗	✓	n/a	n/a	+	
Liver failure	319 (2)	Omitted	1	✓	✗	✗	✗	✓	n/a	n/a	+	
Mortality	702 (3)	OR 1.21 (0.23 to 6.35)	1	✓	✗	✓	✗	✓	n/a	n/a	+	
Liver-related morbidity	147 (2)	Omitted	1	✓	✗	✗	✗	✓	n/a	n/a	+	

SD, sinusoidal dilatation; CRLM, colorectal liver metastases; No, number; CI, confidence interval; OR, odds ratio; n/a, not applicable; ✓, no serious limitations; ✗, serious limitations (-1 point); ✗✗, very serious limitations (-2 points); +++, high quality; ++, moderate quality; +, low quality; +, very low quality.

Appendix 2.6. Modified grade summary of findings table for the influence of sd grade 1-3 vs. sd grade 0 on outcome after partial hepatectomy

A table depicting the influence of sinusoidal dilatation (SD) grade 1-3 versus SD grade 0 on morbidity, liver failure, mortality, and liver-related morbidity after partial liver resection. The grading of recommendations assessment, development and evaluation tool (GRADE) is applied per outcome and a subsequent rationale for the overall GRADE assessment score is given

Patient or population: patients with CRLM Setting: hospital Prognostic factor: SD grade 1-3				
Outcomes	Number of participants	Number of studies	Estimated effect size (95% CI)	GRADE assessment ¹
Morbidity	577	3	OR 1.32 (0.98 to 1.77)	Very low ^{2,3,6}
Follow-up: 30 to 90 days or in-hospital				
Liver failure	0	0	Not estimable	n/a
Follow-up: 30 to 90 days or in-hospital				
Mortality	433	2	OR 0.52 (0.06 to 4.25)	Very low ^{4,6}
Follow-up: 30 to 90 days or in-hospital				
Liver-related morbidity	473	2	OR 1.43 (0.61 to 3.35)	Very low ^{2,3,4,6}
Follow-up: 30 to 90 days or in-hospital				

¹ In the exemplar review for prognostic studies of Hayden et al.³⁰, phase 2 and 3 explanatory studies start with a high grade score (four points), while phase 1 explanatory studies start with a moderate score (three points). Since all outcomes consisted for ≥50% of Phase 1 studies, the starting score for all outcomes was set on three points by the authors of this review.² The definition of the outcome was not clearly stated in all included articles or there were differences in outcome definition between the included studies for this outcome;³ The time period for measurement of the outcome was not clearly stated in ≥50% of the included studies;⁴ (Almost) no overlap in intervals and estimate points of effect could be found on both sides of the null line in meta-analysis for this outcome;⁵ There was significant unexplained heterogeneity for this outcome as defined by an I²≥65% and a p-value <0.10;⁶ This outcome contains imprecise results, defined as an inclusion of only two studies and/or an insufficient sample size, wide confidence intervals or confidence intervals crossing the null value in ≥50% of studies.

GRADE, Grading of Recommendations Assessment, Development and Evaluation; SD, sinusoidal dilatation; CRLM, colorectal liver metastases; CI, confidence interval; OR, odds ratio; n/a, not applicable; +, low quality; ++, moderate quality; +++, high quality; +++, high quality; +, very low quality.

Appendix 2.7. Detailed quality assessment of all outcomes with the grading of recommendations assessment, development and evaluation tool (grade)

The influence of sinusoidal dilatation (SD) grade 1-3 versus SD grade 0 on morbidity, liver failure, mortality, and liver-related morbidity after partial hepatectomy is depicted. Per outcome, specific domains of the grading of recommendations assessment, development and evaluation tool (GRADE) are scored and a subsequent overall score is calculated

The influence of SD grade 1-3 compared to SD grade 0 on short-term outcome after partial hepatectomy											
Patient or population: patients with CRLM											
Setting: hospital											
Prognostic factor: SD grade 1-3											
			GRADE factors								
Outcomes	No. of participants (studies)	Estimated effect size (95% CI)	Phase	Study limitations	Inconsistency	Indirectness	Imprecision	Publication bias	Moderate/large effect size	Dose effect	Overall score
Morbidity	577 (3)	RR 1.53 (0.96 to 2.44)	1	✓	✓	✗	✗	✓	n/a	n/a	+
Liver failure	0	Not estimable	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Mortality	433 (2)	RR 0.51 (0.06 to 4.38)	1	✓	✗	✓	✗	✓	n/a	n/a	+
Liver-related morbidity	473 (2)	RR 2.22 (0.34 to 14.32)	1	✓	✗	✗	✗	✓	n/a	n/a	+

SD, sinusoidal dilatation; CRLM, colorectal liver metastases; No, number; CI, confidence interval; RR, risk ratio; n/a, not applicable; ✓, no serious limitations; ✗, serious limitations (-1 point); ✗✗, serious limitations (-2 points); +++, high quality; ++, moderate quality; +, low quality; +, very low quality.

Chapter 3

Systematic review of the influence of chemotherapy-associated liver injury on outcome after partial hepatectomy for colorectal liver metastases

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Abstract

Background

The impact of chemotherapy-associated liver injury (CALI) on postoperative outcome in patients undergoing partial hepatectomy for colorectal liver metastases (CRLM) remains controversial. The objective of this study was to clarify the effect of CALI (sinusoidal dilatation (SD), steatosis, and steatohepatitis) on postoperative morbidity and mortality by investigating a large dataset from multiple international centres.

Methods

Pubmed and Embase were searched for studies published between 1 January 2004 and 31 December 2013 with keywords: “chemotherapy”, “liver resection”, “outcome”, and “colorectal metastases” to identify potential collaborating centres. Univariable and multivariable analyses were performed using binary logistic regression models, with results presented as odds ratio (ORs) with 95% confidence intervals (CIs).

Results

A consolidated database comprising 788 patients who underwent hepatectomy for CRLM in eight centres was obtained. In multivariable analyses, severe SD was associated with increased major morbidity (Dindo-Clavien grade III-V; OR 1.73, 95% CI 1.02-2.95, $p=0.043$). Severe steatosis was associated with decreased liver surgery-specific complications (OR 0.52, 95% CI 0.27-1.00, $p=0.049$), whereas steatohepatitis was linked to an increase in these complications (OR 2.08, 95% CI 1.18-3.66, $p=0.012$). Subgroup analysis showed that lobular inflammation was the sole component associated with increased overall morbidity (OR 2.22, 95%CI 1.48-3.34, $p=0.001$) and liver surgery-specific complications (OR 3.35, 95%CI 2.11-5.32, $p<0.001$). Finally, oxaliplatin treatment was linked to severe SD (OR 2.74, 95%CI 1.67-4.49, $p<0.001$).

Conclusions

An increase in postoperative major morbidity and liver surgery-specific complications was observed after partial hepatectomy in patients with severe SD and steatohepatitis. Moreover, postoperative liver failure occurred more often in patients with severe SD.

Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide, affecting over 1.3 million patients annually.¹ Approximately 50% of these patients develop colorectal liver metastases (CRLM).^{2,3} Although liver resection provides the best prospect of cure, only 10-30% of patients with liver metastases are eligible for hepatic surgery.^{3,4} For patients with tumours deemed unresectable, neoadjuvant systemic chemotherapy can prolong survival, and allow future hepatic resection.^{2,5}

For decades, 5-fluorouracil (5-FU) was the sole option for treating CRLM. This has changed markedly in the new millennium; with the approval of irinotecan, oxaliplatin, and humanized monoclonal antibodies, approximately 15% of patients with initially unresectable tumours became eligible for liver resection.^{6,7} Unfortunately, administration of irinotecan- and/or oxaliplatin-based chemotherapeutic agents has been associated with a harmful side-effect in the form of liver injury.^{8,9}

Chemotherapy-associated liver injury (CALI) is often reported in patients with CRLM, and appears to be regimen specific. For instance, oxaliplatin treatment is associated with sinusoidal obstruction syndrome (SOS),¹⁰ and linked to increased occurrence of nodular regenerative hyperplasia (NRH).¹¹ Co-administration of bevacizumab with oxaliplatin, however, has been reported to be associated with a decrease in both the incidence and severity of SOS and NRH.¹⁰⁻¹² Irinotecan-based regimens appear to be related to the development of steatohepatitis.^{8,13,14} Importantly, because patients commonly receive several chemotherapeutic agents to offer optimal benefit in downsizing tumours, it is difficult to identify the specific agents responsible for injury to the hepatic parenchyma.

Certain studies^{9,13,15-18} have claimed a negative correlation between CALI and postoperative outcome (postoperative morbidity, mortality), but others¹⁹⁻²³ could not reproduce this. Therefore, it remains unclear whether CALI influences postoperative morbidity and mortality. The aim of the present study was to explore whether sinusoidal dilatation (SD), steatosis, and steatohepatitis are associated with increased morbidity and mortality rates after partial hepatectomy by performing a meta-analysis of individual participant data based on a systematic literature review. Additionally, factors associated with the occurrence of CALI were identified.

Methods

An extensive protocol, written before the start of this study, can be found in Appendix 3.1. PRISMA and Moose guidelines were followed when conducting and reporting this review.^{24,25}

Search strategy for identification of studies

Systematic searches were performed in MEDLINE (PubMed) and Embase for studies published between 1 January 2004 and 31 December 2013 using a search matrix including the following four categories: liver resection, chemotherapy, tumour type, and outcome. For the purpose of performing a more comprehensive search, the type of liver injury was not included in the searching matrix. The full search strategy is shown in the supplemental data (Appendix 3.1). The first publication date was fixed at 2004 because the widely used criteria for scoring SD, steatosis, and steatohepatitis were developed in 2004²⁶ and 2005.²⁷ No language filter was applied.

Inclusion criteria

Studies meeting the following criteria were considered eligible for inclusion: adult patients (aged over 18 years), who underwent liver resection for CRLM, with description of postoperative short-term overall morbidity, liver surgery-specific complications, postoperative liver failure, or overall mortality (within 90 days or in-hospital) after liver resection, and with pathological assessment of non-tumorous liver specimens for SD, steatosis, and/or steatohepatitis (Appendix 3.1). Studies with patients who received preoperative hepatic arterial infusion of chemotherapy were excluded, as were case reports, comments, those with published abstracts only, editorials and reviews.

Study identification and data collection

Duplicates were removed. Two authors independently screened all titles and abstracts and excluded those not pertinent to the review. Any discrepancy was solved by consensus. The remaining articles were included for full-text revision and assessed independently for eligibility by the two authors. Reference lists of full-text reviewed articles were checked manually for additional potential citations, along with exploration of a personal library that was established during previous research on this topic.^{28,29}

Definitions

SD was graded according to Rubbia-Brandt and colleagues,²⁶ with grade 2-3 (severe SD) considered clinically relevant since it reflects rupture of sinusoidal wall integrity. SD is one of the most important histological features of SOS, and its severity is generally accepted to correspond to the severity of SOS. Steatosis and steatohepatitis were graded according to Kleiner et al.²⁷ Severe steatosis was defined as more than 33% of parenchyma affected by steatosis.²⁷ A non-alcoholic steatohepatitis activity score (NAS) of 4 or more was considered as steatohepatitis to provide a working cut-off value conforming with the literature.³⁰ CALI was defined as any occurrence of the severe SD, severe steatosis, or steatohepatitis. Co-morbidity was defined as any disease affecting the patient apart from colorectal liver metastases (diabetes mellitus, pulmonary, renal, cardiovascular and other diseases). Overall morbidity was defined as any complication occurring within 90 days after surgery or during the hospital stay, and graded according to the classification of Dindo and colleagues.³¹ Major morbidity was defined as Dindo-Clavien grade III (requiring invasive intervention) or higher. The concept of a liver surgery-specific complication was in correspondence to the liver surgery-specific composite endpoint developed in 2011, and included one or more of the following events: ascites, postoperative liver failure, bile leakage, intra-abdominal abscess, intra-abdominal haemorrhage, and operative mortality.³² Postoperative liver failure was defined as the concurrent presence of a prothrombin time below 50% and a serum bilirubin greater than 50 µM/L (the 50-50 criteria) on or after postoperative day 5.³³ Postoperative mortality included death from any cause within 90 days after surgery or during hospital admission. Major hepatectomy was defined as resection of three or more Couinaud liver segments.³⁴

Risk-of-bias assessment

The risk of bias of the final included studies was assessed independently by two blinded researchers using the quality in prognosis studies (QUIPS) tool.^{35,36} Discrepancies were discussed by the two authors and consensus was reached. The QUIPS tool includes six bias domains: participation, attrition, prognostic factor measurement, confounding measurement and account, outcome measurement, and analysis and reporting. The results of each of the six domains were taken together to calculate an overall low, moderate, or high risk of bias, as follows: overall low risk of bias, two or fewer domains rated as moderate risk and the remaining domains as low risk; overall moderate risk, at least three domains rated as moderate risk and the remaining domains as low risk; and high overall risk, one or more domain rated as high risk, independent of the rating of the remaining domains.

Data handling and statistical methods

Corresponding authors from studies that fulfilled the inclusion criteria were contacted by email for collaboration and sharing of coded data (by numbering) for the published cohort. Each author was asked to sign a specific data transfer agreement form, which assured careful handling of the data. Coded data were arranged in a preconstructed Excel™ file (Microsoft, Redmond, Washington, USA) and subsequently imported into SPSS® for Windows® version 20.0 (IBM, Armonk, New York, USA).

Patient characteristics were compared using the Student's *t* test for numerical variables. For categorical variables, the Pearson Chi-square test with continuity correction was applied, or Fisher's exact test when any of the expected values was smaller than five. The influence of preoperative chemotherapeutic agents on liver injury, and subsequently the effect of liver injury on short-term postoperative outcome were analysed applying one-step binary logistic regression models. Individual participant data from all studies were pooled and modelled simultaneously. This approach was considered more optimal because each study showed relatively few events per outcome and small sample sizes, and the one-step approach for pooled data allowed the exact binomial distribution to be used and did not require continuity corrections when there were no events.^{37,38} For missing values, multiple imputations were performed, assuming that data were missing at random. The number of imputations was determined by the maximum percentage of missing data in the data set. In this study, 30 imputations were performed, as the maximum amount of missing data was 26% (minor/major morbidity). Variables in multiple imputations are listed in Appendix 3.2. A complete-case sensitive analysis was also conducted. Clustering of patients from different studies was integrated as a separate co-variable ('database source') and included in binary logistic regression models in every analysis.³⁸ A single variable together with database source created the univariable model. All variables with $p \leq 0.200$ in univariable analysis were included in the multivariable analysis. In addition, database source and variables known to be related to the outcome (either well-described in literature or based on careful discussion and consensus between the authors) were forced into the multivariable model.

A subgroup analysis including only patients who received oxaliplatin-based treatment was performed to investigate the impact of bevacizumab on the occurrence of severe SD. At a later stage of the study, data to investigate the influence of separate NAS-subcategories (steatosis, lobular inflammation and hepatocellular ballooning) on postoperative outcome were requested. A subgroup analysis including cohorts with available data on NAS-subcategories was undertaken. The methods of analysis of these data were identical to those described above. Unadjusted and adjusted odds ratios (ORs)

with 95% confidence intervals (95% CIs) were calculated. $p \leq 0.050$ was considered significant for all analyses.

Results

The search strategy resulted in 1191 unique hits (Figure 3.1). In total, 1093 articles were excluded on the base of title or abstract, and the remaining 98 articles were subjected to full-text evaluation. Thirty-two studies met the inclusion criteria and the 30 corresponding authors were contacted by e-mail. Nineteen authors responded, of whom eight agreed to share their raw data. Other authors could not be contacted (Appendix 3.3). Potential publication bias was excluded by testing for asymmetry in a funnel plot. The eight included studies^{16,17,39-44} were assessed for risk of bias using the QUIPS tool. Overall ratings are shown in Table 3.1. Six articles showed an overall low risk of bias and one study each showed a moderate or high risk. Detailed scorings can be found in Appendix 3.4. Because the individual participant data from all studies were pooled and modelled simultaneously, an overall rating was also calculated without taking the study confounding and statistical analysis domains into account; all studies then showed a low risk of bias based on the remaining four domains.

Characteristics of the included studies are summarized in Table 3.1. The consolidated cohort consisted of 816 patients (Figure 3.1). Twenty-eight patients were excluded because they underwent surgery for indications other than CRLM, leaving 788 patients for analysis. Patient characteristics, surgical details, and postoperative outcomes of the consolidated cohort are listed in Table 3.2. There were 453 men (57.5%) and 335 women (42.5%) with a median age of 61 (range 25-86) years. SD was severe in 183 patients (24.1%), steatosis was severe in 117 (15.6%), and steatohepatitis in 100 (14.5%). Of the 525 patients who received 5-FU, 396 (75.4%) had simultaneous treatment with oxaliplatin and 135 (25.7%) with irinotecan. Of the 136 patients who received capecitabine, 119 (87.5%) had simultaneous treatment with oxaliplatin. Cetuximab and bevacizumab were most frequently administered together with oxaliplatin. All 61 patients who received cetuximab, and 138 of 164 (84.1%) patients who received bevacizumab, were co-treated with oxaliplatin. Of 635 patients with NAS subcategory data, 96 (15.1%) had a NAS of 4 or more. Only three patients (3.1%) with a NAS of 4 or higher did not show lobular inflammation. The relationship between NAS and lobular inflammation is summarized in Appendix 3.5.

Figure 3.1 Selection process of included articles.
CTx, chemotherapy; SD, sinusoidal dilatation; CALI, chemotherapy-associated liver injury; PH, partial hepatectomy; HCC, hepatocellular carcinoma; CCA, cholangiocarcinoma.

Table 3.1 Characteristics of studies included in final database.

Reference	Year	Country	Study type	Population*	QUIPS Score	Comparisons	Key findings
Gomez-Ramirez ⁶⁰	2010	Spain	PCS	45 (46)	Low	Neoadjuvant CTx vs. no CTx	Patients treated with oxaliplatin had a higher incidence of SOS, an increase in liver complications and longer mean hospital stay
Nam ⁶¹	2011	Korea	RCS	89	Low	Oxaliplatin CTx vs. non-oxaliplatin CTx	Sinusoidal injury is frequently seen in oxaliplatin-treated livers and should be documented in surgical pathology practice when extensive
Pessaux ⁶²	2010	France	RCC	52 (36)	High	Neoadjuvant CTx vs. CTx + cetuximab/bevacizumab	The addition of bevacizumab or cetuximab to the neoadjuvant chemotherapy does not increase the morbidity rates after hepatectomy for CRLM
Pilgrim ¹⁶	2012	Australia	RCS	232	Low	Severe vs. non-severe CALI	Severe steatosis was associated with increased postoperative morbidity, while severe steatohepatitis and sinusoidal injury were not
Soubrane ⁶³	2010	France	RCS	78 (105)	Low	Severe vs. non-severe SD	SOS 2-3 was associated with postoperative hepatic dysfunction and ascites after major liver resection
Takamoto ⁶⁴	2010	Japan	RCS	55 (104)	Moderate	Liver injury vs. no liver injury	The hepatic functional reserve, represented by the ICG R15 value, improves during the period after chemotherapy cessation
Van der Pool ⁴⁸	2012	Netherlands	RCS	104	Low	Neoadjuvant CTx vs. CTx + Bevacizumab	Bevacizumab added to oxaliplatin-based chemotherapy may protect against moderate sinusoidal dilatation without significantly influencing morbidity
Vigano ¹⁷	2012	Italy	PCS	100	Low	Severe vs. non-severe CALI	Liver biopsy cannot be considered a reliable tool in assessing CALI except for steatosis. Proportion of liver dysfunction was higher among patients with CALI

*Values are number of patients in cohort reported in published article, with number in shared database in parentheses. QUIPS, Quality in Prognosis Studies; PCS, prospective cohort study; CTx, chemotherapy; SOS, sinusoidal obstruction syndrome; RCS, retrospective cohort study; RCC, retrospective case-control study; CRLM, colorectal liver metastases; CALI, chemotherapy-associated liver injury; SD, sinusoidal dilatation; ICG-R15, indocyanine green retention rate at 15 min.

Table 3.2 Patient, surgical, and postoperative characteristics.

	Number of patients	Sinusoidal dilatation (n=760)			Steatosis (n=752)			Steatohepatitis (n=690)		
		Non-severe (n=577)	Severe (n=183)	Unadjusted p-value*	Non-severe (n=635)	Severe (n=117)	Unadjusted p-value*	NAS <4 (n=590)	NAS ≥4 (n=100)	Unadjusted p-value*
Gender				0.836			1.000 [†]			0.784 [†]
Male	453	335 (58.1%)	104 (56.8%)		366 (57.6%)	68 (58.1%)		336 (56.9%)	59 (59.0%)	
Female	335	242 (41.9%)	79 (43.2%)		269 (42.4%)	49 (41.9%)		254 (43.1%)	41 (41.0%)	
Mean age (years)	788	61 (SD 11)	60 (SD 10)	0.576 [†]	61 (SD 11)	60 (SD 9)	0.527 [†]	61 (SD 11)	61 (SD 10)	0.784 [†]
Mean BMI (kg/m ²)	706	25.3 (SD 4.4)	24.4 (SD 3.9)	0.001 [†]	24.7 (SD 4.1)	27.6 (SD 4.1)	<0.001 [†]	24.9 (SD 4.2)	26.7 (SD 3.9)	<0.001 [†]
Comorbidity				0.259 [†]			0.012 [†]			0.158 [†]
No	363	247 (49.3%)	93 (54.7%)		290 (52.2%)	41 (38.3%)		271 (51.4%)	31 (41.9%)	
Yes	336	254 (50.7%)	77 (45.3%)	0.001 [†]	266 (47.8%)	66 (61.7%)	0.032 [†]	256 (48.6%)	43 (58.1%)	0.198 [†]
Preoperative chemotherapy										
No	127	110 (19.3%)	15 (8.4%)		114 (18.2%)	11 (9.6%)		112 (19.3%)	13 (13.3%)	
Yes	649	459 (80.7%)	164 (91.6%)	0.006 [†]	511 (81.8%)	104 (90.4%)	0.463 [†]	468 (80.7%)	85 (86.7%)	0.396 [†]
5-FU										
No	245	201 (35.6%)	43 (24.2%)		210 (33.9%)	34 (29.8%)		213 (37.0%)	31 (32.0%)	
Yes	525	363 (64.4%)	135 (75.8%)	0.173 [†]	408 (66.1%)	80 (70.2%)	0.158 [†]	362 (63.0%)	66 (68.0%)	0.061 [†]
Capecitabine										
No	634	454 (80.5%)	152 (85.4%)		511 (82.4%)	87 (76.3%)		466 (81.0%)	70 (72.2%)	
Yes	136	110 (19.5%)	26 (14.6%)	0.033 [†]	109 (17.6%)	27 (23.7%)	0.033 [†]	109 (19.0%)	27 (27.8%)	1.000 [†]
Irinotecan										
No	627	445 (78.9%)	154 (86.5%)		508 (81.9%)	83 (72.8%)		453 (78.8%)	77 (79.4%)	
Yes	143	119 (21.1%)	24 (13.5%)	0.072 [†]	112 (18.1%)	31 (27.2%)	0.143 [†]	122 (21.2%)	20 (20.6%)	0.101 [†]
Bevacizumab										
No	605	430 (76.4%)	148 (83.1%)		487 (78.7%)	82 (71.9%)		444 (77.4%)	67 (69.1%)	
Yes	164	133 (23.6%)	30 (16.9%)	0.198 [†]	132 (21.3%)	32 (28.1%)	0.716 [†]	130 (22.6%)	30 (30.9%)	0.462 [†]
Cetuximab										
No	708	522 (92.7%)	159 (89.3%)		566 (91.4%)	106 (93.0%)		522 (90.9%)	91 (93.8%)	
Yes	61	41 (7.3%)	19 (10.7%)	<0.001 [†]	53 (8.6%)	8 (7.0%)	0.716 [†]	52 (9.1%)	6 (6.2%)	0.295 [†]
Oxaliplatin										
No	266	228 (40.5%)	37 (20.8%)		226 (36.5%)	39 (34.2%)		231 (40.2%)	33 (34.0%)	(continued)
Yes	503	335 (59.5%)	141 (79.2%)		393 (63.5%)	75 (65.8%)		343 (59.8%)	64 (66.0%)	

Table 3.2 (continued)

	Number of patients	Sinusoidal dilatation (n=760)			Steatosis (n=752)			Steatohepatitis (n=690)		
		Non-severe (n=577)	Severe (n=183)	Unadjusted p-value*	Non-severe (n=635)	Severe (n=117)	Unadjusted p-value*	NAS <4 (n=590)	NAS ≥4 (n=100)	Unadjusted p-value*
Resection type										
Minor (<3 segments)	398	330 (57.2%)	67 (36.8%)	<0.001 [†]	335 (52.8%)	63 (53.8%)	0.921 [†]	339 (57.6%)	55 (55.0%)	0.713 [†]
Major (≥3 segments)	389	247 (42.8%)	115 (63.2%)		299 (47.2%)	54 (46.2%)		250 (42.4%)	45 (45.0%)	
PVE										
No	623	461 (92.0%)	140 (82.4%)	0.001 [†]	498 (89.6%)	103 (96.3%)	0.046 [†]	491 (93.2%)	71 (95.9%)	0.512 [†]
Yes	76	40 (8.0%)	30 (17.6%)		58 (10.4%)	4 (3.7%)		36 (6.8%)	3 (4.1%)	
Pringle Maneuver										
No	446	347 (64.5%)	93 (54.1%)	0.018 [†]	366 (62.1%)	71 (62.8%)	0.974 [†]	349 (63.9%)	61 (64.2%)	1.000 [†]
Yes	291	191 (35.5%)	79 (45.9%)		223 (37.9%)	42 (37.2%)		197 (36.1%)	34 (35.8%)	
Transfusion of packed RBCs										
No	646	492 (86.2%)	139 (76.8%)	0.004 [†]	529 (84.4%)	100 (85.5%)	0.871 [†]	508 (86.8%)	83 (83.0%)	0.382 [†]
Yes	134	79 (13.8%)	42 (23.2%)		98 (15.6%)	17 (14.5%)		77 (13.2%)	17 (17.0%)	
Postoperative short-term outcome										
Mean hospital stay (days)	768	14 (SD 13)	15 (SD 11)	0.240 [†]	15 (SD 11)	14 (SD 17)	0.660 [†]	14 (SD 12)	15 (SD 14)	0.266 [†]
Overall morbidity										
No	443	339 (59.3%)	87 (47.8%)	0.009 [†]	360 (57.3%)	66 (56.4%)	0.935 [†]	355 (60.6%)	48 (48.0%)	0.024 [†]
Yes	338	233 (40.7%)	95 (52.2%)		268 (42.7%)	51 (43.6%)		231 (39.4%)	52 (52.0%)	
None/minor morbidity (DCO-II)	491	362 (88.5%)	106 (73.1%)	<0.001 [†]	397 (85.6%)	69 (85.2%)	1.000 [†]	367 (89.5%)	62 (80.5%)	0.041 [†]
Major morbidity (DC II-V)	89	47 (11.5%)	39 (26.9%)		67 (14.4%)	12 (14.8%)		43 (10.5%)	15 (19.5%)	
Liver surgery-specific complication										
No	598	455 (79.8%)	121 (66.9%)	<0.001 [†]	475 (75.9%)	100 (85.5%)	0.031 [†]	470 (80.6%)	75 (75.0%)	0.247 [†]
Yes	180	115 (20.2%)	60 (33.1%)		151 (24.1%)	17 (14.5%)		113 (19.4%)	25 (25.0%)	
Mortality										
No	780	573 (99.3%)	179 (97.8%)	0.200 [§]	628 (98.9%)	116 (99.1%)	1.000 [§]	586 (99.3%)	98 (98.0%)	0.422 [§]
Yes	8	4 (0.7%)	4 (2.2%)		7 (1.1%)	1 (0.9%)		4 (0.7%)	2 (2.0%)	
Postoperative liver failure										
No	755	563 (98.6%)	166 (91.7%)	<0.001 [†]	608 (97.0%)	116 (99.1%)	0.302 [§]	578 (99.0%)	99 (99.0%)	1.000 [§]
Yes	24	8 (1.4%)	15 (8.3%)		19 (3.0%)	1 (0.9%)		6 (1.0%)	1 (1.0%)	

SD, standard deviation; NAS, non-alcoholic fatty liver diseases activity score; BMI, body mass index; 5-FU, fluorouracil; PVE, portal venous embolization; RBCs, red blood cells; DC, Dindo-Clavien; *p-value was not adjusted by database source; † Student's t-test; ‡ Pearson Chi-Square test with continuity correction; §Fisher's exact test; data represent original data without multiple imputations; due to missing values, some numbers do not add up to the total number of patients.

Sensitivity analysis showed similar results between complete case analysis and that with multiple imputations (detailed information available on request). The influence of severe SD, severe steatosis, steatohepatitis, and other potential factors related to short-term overall morbidity, liver surgery-specific complications, and major morbidity is shown in Figure 3.2 and Appendix 3.6. Severe SD was significantly associated with increased major morbidity only (OR 1.73, 95% CI 1.02-2.95, $p=0.043$). Severe steatosis was not significantly associated with the occurrence of postoperative overall or major morbidity, but was related to a decreased occurrence of liver surgery-specific complications (OR 0.52, 95% CI 0.27-1.00, $p=0.049$). In contrast, patients with steatohepatitis showed a significantly increased rate of postoperative liver surgery-specific complications (OR 2.08, 95% CI 1.18-3.66, $p=0.012$), and a trend towards increased overall morbidity (OR 1.58, 95% CI 0.99-2.52, $p=0.057$).

Figure 3.2 Multivariable analysis of influence of liver injury on short-term postoperative outcomes. Data are depicted as OR (symbol) with 95% confidence intervals (bars) based on results of multivariable analysis; BMI, body mass index; PVE, portal venous embolization; Ctx, chemotherapy; SD, sinusoidal dilatation; asterisks indicate $p\text{-values} \leq 0.05$.

Table 3.3 summarizes the effects of several factors associated with postoperative liver failure and mortality. Because of the small number of events, multivariable logistic regression was not performed. Postoperative liver failure occurred in 24 of 779 patients (3.1%) in the study cohort (Table 3.2), seven women and 17 men, with a median age of 61 (range 48-75) years. All patients with liver failure had undergone major hepatectomy. The only factors strongly associated with increased liver failure in univariable analysis

were severe SD (OR 3.06, 95% CI 1.18-7.92, $p=0.021$) and perioperative blood transfusion (OR 4.47, 95% CI 1.69-11.82, $p=0.003$).

Table 3.3 Univariable analyses of factors influencing short-term postoperative outcome.

Variable	Postoperative liver failure ($n=24$)		Mortality ($n=8$)	
	OR [95% CI]	p-value	OR [95% CI]	p-value
Gender (female)	0.65 [0.25-1.68]	0.376	0.50 [0.10-2.25]	0.408
Age (years)	1.00 [0.96-1.04]	0.937	1.03 [0.96-1.05]	0.402
BMI (kg/m^2)	0.90 [0.80-1.01]	0.070	0.96 [0.80-1.14]	0.630
Comorbidity	1.28 [0.53-3.13]	0.583	7.84 [0.92-66.65]	0.059
PVE	2.62 [0.95-7.26]	0.064	1.09 [0.13-9.09]	0.938
Preoperative chemotherapy	2.41 [0.30-19.32]	0.409	1.51 [0.52-4.42]	0.702
Resection type (major)	n/a [†]	n/a [†]	6.56 [0.77-55.90]	0.085
Pringle manoeuvre	1.45 [0.57-3.72]	0.437	1.91 [0.43-8.54]	0.399
Blood transfusion	4.47 [1.69-11.82]	0.003	14.00 [2.74-71.51]	0.002
Severe SD	3.06 [1.18-7.92]	0.021	2.79 [0.68-11.44]	0.155
Severe steatosis	0.47 [0.06-3.77]	0.473	0.86 [0.10-7.09]	0.887
Steatohepatitis	1.90 [0.31-22.83]	0.492	2.71 [0.57-12.87]	0.210

BMI, body mass index; PVE, portal venous embolization; SD, sinusoidal dilatation; OR, odds ratio; 95% CI, 95% confidence intervals; n/a, not applicable; Multiple imputations were used for univariable analysis; [†]the proportion of patients with liver failure is 100% for patients with major hepatectomy and 0% for those with minor hepatectomy (Fisher's exact test: $p < 0.001$)

In total, eight patients (1.0%) died in the perioperative period after liver resection. Seven deaths happened within 90 days and one occurred at 101 days during hospital stay. The six men and two women had a median age of 64 (range 48-75) years. Perioperative blood transfusion (OR 14.00, 95% CI 2.74-71.51, $p=0.002$) was the sole factor related to increased mortality in univariable analysis. A trend was found for major liver resection (OR 6.56, 95% CI 0.77-55.90, $p=0.085$) and preoperative comorbidity (OR 7.84, 95% CI 0.92-66.65, $p=0.059$) as factors associated with increased postoperative mortality. Severe SD (OR 2.79, 95% CI 0.68-11.44, $p=0.155$), severe steatosis (OR 0.86, 95% CI 0.10-7.09, $p=0.887$), and steatohepatitis (OR 2.71, 95% CI 0.57-12.87, $p=0.210$) were not related to postoperative mortality.

Because steatohepatitis, but not severe steatosis, negatively affected postoperative short-term outcomes, lobular inflammation and hepatocellular ballooning were considered key factors for poor outcome. A subgroup analysis supported this hypothesis. In multivariable analyses, severe (grade 2-3) lobular inflammation was associated with increased overall postoperative morbidity (OR 2.22, 95% CI 1.48-3.34, $p=0.001$) and liver surgery-specific morbidity (OR 3.35, 95% CI 2.11-5.32, $p<0.001$), but not major morbidity (OR 1.63, 95% CI 0.85-3.10, $p=0.138$). In contrast, neither severe steatosis (>33%) nor

the presence of hepatocellular ballooning (grade 1-2) were associated with an increased complication rate in all multivariable analyses.

Associations between several preoperative variables and the occurrence of severe SD, severe steatosis, and steatohepatitis are summarized in Figure 3.3 and Appendix 3.7. Oxaliplatin (OR 2.74, 95% CI 1.67-4.49, $p<0.001$) was related increased occurrence of severe SD in multivariable analysis, whereas the addition of bevacizumab was related to a twofold decrease in the occurrence of severe SD in patients who received oxaliplatin (OR 0.50, 95% CI 0.30-0.82, $p=0.006$) when the analysis was adjusted solely by database source. Patients with severe steatosis showed a decreased incidence of severe SD (OR 0.44, 95% CI 0.24-0.83, $p=0.011$) and *vice versa* (OR 0.36, 95% CI 0.15-0.88, $p=0.025$). BMI was related to an increase in severe steatosis (OR 1.15, 95% CI 1.08-1.21, $p<0.001$), whereas the incidence of severe steatosis was decreased in patients with portal venous embolization (OR 0.29, 95% CI 0.08-1.00, $p=0.050$). Only severe steatosis was significantly associated with an increased occurrence of steatohepatitis (OR 15.09, 95% CI 6.25-36.45, $p<0.001$).

Figure 3.3 Multivariable analysis of influence of risk factors on liver injury. Data are depicted as OR (symbol) with 95% confidence intervals (bars) based on results of multivariable analysis; BMI, body mass index; PVE, portal venous embolization; 5-FU, 5-fluorouracil; SD, sinusoidal dilatation; asterisks indicate p -values ≤ 0.05 .

Discussion

In this study, an increase in postoperative major morbidity and liver surgery-specific complications after partial hepatectomy in patients with sinusoidal dilatation and steatohepatitis were observed, whereas steatosis was associated with a decreased occurrence of complications. Moreover, postoperative liver failure occurred more often in patients with severe SD. With respect to steatohepatitis, lobular inflammation, but not severe steatosis and hepatocellular ballooning, was strongly linked to increased postoperative morbidity. Oxaliplatin-based chemotherapy was the sole factor independently associated with an increase in the occurrence of severe SD, whereas a decrease in the occurrence of severe SD was seen with the addition of bevacizumab. Moreover, an inverse relationship between severe SD and severe steatosis was found.

Mechanisms for the negative influence of severe SD on major morbidity are unknown, although preoperative hepatic dysfunction, impairment of liver regeneration, Kupffer cell dysfunction, enhanced blood loss due to haemorrhagic pools, fragility of the liver, and increased hepatocellular necrosis as seen in human and animal models can all be reasons for poor liver function and an increased complication rate after severe SD.^{39,40} However, since the p-value was borderline significant, the effect of severe SD on major morbidity must be interpreted with caution.

The progression of steatohepatitis is estimated by the so-called NAS score, which is composed of scores for steatosis, lobular inflammation, and hepatocellular ballooning.²⁷ Multivariable subgroup analyses concerning the influence of these separate NAS-subcategories on postoperative outcome showed a detrimental influence of lobular inflammation on all outcomes, whereas severe steatosis and hepatocellular ballooning had no effect. The mechanism behind steatohepatitis being discriminant for postoperative short-term outcomes is uncertain. Electron microscopy revealed that mitochondrial structural defects in hepatocytes are correlated with steatohepatitis, but not with steatosis.⁴¹ Moreover, the ability of the liver to recover from ATP depletion was severely impaired,⁴² liver regeneration was diminished,⁴³ and humoral and cellular immune responses in reaction to enhanced oxidative stress were found in patients with steatohepatitis. These factors may be accountable for postoperative complications.

Severe steatosis did not significantly influence short-term overall morbidity, major morbidity, or mortality after partial hepatectomy in this study, which is in line with previous reports^{44,45} but in contrast to other studies.^{46, 47} Patients with severe steatosis even showed a decreased occurrence of liver surgery-specific complication. This might

be explained by surgeons being more careful during surgery when observing a severely steatotic (“yellow”) liver. However, with a p-value of 0.049, this evidence needs to be validated by future research.

Patients who received oxaliplatin showed an increased occurrence of severe SD compared to patients who did not receive oxaliplatin (29.6% vs. 14.0%). This is in line with former studies which showed a similarly high occurrence of severe SD after oxaliplatin-based regimens.^{10,17} The addition of bevacizumab, an angiogenesis inhibitor, to oxaliplatin-based chemotherapy has been associated with a decreased incidence of SD.⁴⁸ Indeed, when the analysis was restricted to the population that received oxaliplatin-based treatment, bevacizumab was associated with a remarkably decreased occurrence of severe SD. Although mechanisms underpinning those observations remain unclear, activation of vascular endothelial growth factor and coagulation pathways in oxaliplatin-related SD might be accountable.⁴⁹

Importantly, severe steatosis was linked to a decreased occurrence of severe SD and *vice versa*, raising the possibility that these events are mutually exclusive. Several phenomena could underlie this observation. First, mechanical pressure exerted by fat-laden, swollen hepatocytes may distort the hepatic microvasculature. In mice with severe steatosis, a decrease in sinusoidal perfusion, loss of fenestrae, and narrowing of the sinusoidal lumen were observed.⁵⁰ Spatially, sinusoidal dilatation may therefore not develop. Conversely, atrophied hepatocellular plates in severe SD may render these hepatocytes incapable of fatty acid (FA) uptake. Alternatively, histological assessment may be more challenging in a liver affected by both severe SD and steatosis, increasing the likelihood of misclassification of either one of these injury types. However, several independently contacted pathologists with hepatobiliary expertise considered this probability very small. Although the reduction in sinusoidal dilation in patients with severe steatosis is interesting, central pathology review should be performed to verify this finding.

Apart from oxaliplatin, the preoperative performance of PVE showed to be associated with an increased occurrence of severe SD. Previous studies already acknowledged a possible influence of PVE on the development of vascular injury, probably by the induction of ischemia.⁵¹ We hypothesize that the hepatic artery buffer response after PVE might play an even more profound role as shown by the induction of microvascular remodeling and sinusoidal dilatation in the embolized lobe after portal branch ligation in a rodent model.⁵² However, PVE-induced histopathological changes in the embolized

lobe might not be indicative for the non-embolized lobe, and may thus not indubitably affect liver histology and function after resection.⁵³

In the present study it is observed that the occurrence of severe SD is lower in patients receiving additional bevacizumab compared to those receiving oxaliplatin alone. Severe SD was shown to be associated with an increased complication rate and *vice versa*. Although the effect of co-administration with bevacizumab on surgical outcome could not be investigated directly, adding bevacizumab might provide an advantageous effect on postoperative outcome in patients treated with oxaliplatin.

Parenchymal damage due to chemotherapy can be preoperatively diagnosed by radiological and biochemical tools, as reviewed recently in detail by our group.⁵⁴ Despite (experimental) research focusing on CALI, little evidence is available for the treatment of CALI in the human setting.⁵⁴ When liver injury is confirmed preoperatively, surgeons are thus advised to adapt surgical management to prevent complications. In the present study, the transfusion of packed red blood cells was associated with an increased postoperative complication rate, which is in line with previous literature.⁵⁵ Central venous pressure should be low during surgery to prevent excessive blood loss. Moreover, the performance of a major hepatectomy was confirmed to be associated with an increased postoperative complication rate, which encourages minimizing the resection volume. Performing wedge resections instead of a hemihepatectomy, and the use of radiofrequency ablation might be beneficial when feasible.

To our knowledge, this is the first study demonstrating both the effect of chemotherapy on liver injury and the subsequent effect of liver injury on short-term postoperative outcome in a large, multicentre patient cohort. Some limitations of this study should be discussed. NRH has recently caused concern because of its relationship to increased postoperative morbidity.¹¹ Although analysis of the effect of NRH on postoperative outcome would have been of interest, this was not possible due to unavailability of data. Establishment of included databases before or around the year 2013, when NRH did not yet gain the attention it deserves, may be the reason for missing data. With respect to the negative influence of NRH on postoperative outcomes, it is recommended to include NRH in future studies when exploring the relationship between CALI and postoperative outcomes. Next, data on the interval between cessation of chemotherapy and partial hepatectomy, as well as the number of administered cycles, were not available for every study cohort. Therefore, the influence of these factors on the occurrence of CALI and short-term complications could not be evaluated. Next, central review of all histopathology slides would have strengthened the paper substantially. Unfortunately,

this was not feasible due to logistical reasons. It must be highlighted nonetheless, that all sections were reviewed by local pathologists with hepatobiliary expertise and assessed according to uniform, well-established, and globally accepted scoring systems for SD, steatosis, and steatohepatitis. Lastly, since no randomized controlled trials exist on the topic, mainly retrospective cohort studies were included in this review. Despite this limitation, all included studies showed low risk of bias and nearly all original data could be retained, making it the most comprehensive multicentre data cohort currently available.

Conclusion

This study demonstrated that after partial hepatectomy, severe SD and steatohepatitis were related to increased postoperative major morbidity and liver surgery-specific complications. Regarding steatohepatitis, lobular inflammation, but not steatosis or hepatocellular ballooning, was associated with increased postoperative overall morbidity and liver surgery-specific complications. Furthermore, severe SD was linked to an increased occurrence of postoperative liver failure. Lastly, oxaliplatin was strongly related to an increased occurrence of severe SD, and the addition of bevacizumab to oxaliplatin-based regimen reduced the occurrence of severe SD. Considering the negative relationship between CALI and postoperative morbidity, it is advised to adapt surgical management when CALI is diagnosed. Moreover, with decreased chemotherapy responsiveness,^{28,56} shortened overall survival,⁵⁷ and increasing doubts about the usefulness of neoadjuvant chemotherapy in certain patient groups,⁵⁸ one could even speculate that some patients would benefit from immediate resection instead of neoadjuvant chemotherapeutical treatment. Prospective registrations such as the ALPPS-registry⁵⁹ provide a way to obtain a higher level of evidence on this topic.

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Appendix 3.1 Study protocol

Systematic review of the influence of chemotherapy-associated liver injury on outcome after partial hepatectomy for colorectal liver metastases

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3

Summary

Background

Chemotherapeutic regimens (for example oxaliplatin and irinotecan-based), which are commonly administered in patients with colorectal liver metastases (CRLM), have been associated with undesirable so-called chemotherapy-associated liver injury (CALI, sinusoidal dilatation, steatosis and steatohepatitis). The influence of CALI on postoperative outcomes remains controversial.

Objective

We describe the protocol of a systematic review and individual participant data analysis, which aims to assess the impact of CALI on postoperative morbidity and mortality after partial hepatectomy.

Methods

The PRISMA and MOOSE guidelines will be followed for the conduct and report of this review. A literature search will be performed in MEDLINE (PubMed) and Embase, in addition to checking of references in papers found. The search will be limited to studies published between 1 January 2004 and 31 December 2013. No restrictions of language will be applied in the search strategy. We will include studies of any design (except case reports, comments, editorials and reviews) investigating the influence of CALI on postoperative morbidity and mortality within the population of adult patients (aged over 18 years) who underwent partial hepatectomy for colorectal liver metastases. The risk of bias of included papers will be assessed using the Quality in Prognosis Studies (QUIPS) tool for prognosis studies. Corresponding authors of included studies will be contacted for sharing the original data of the patient cohort. The raw data will then be recoded, redefined, pooled and analysed using binary logistic regression models.

Ethics and dissemination

No ethical issues are foreseen because (a) the data from each included study were already published in the literature; (b) each of the included studies obtained local ethics committee approval; (c) the project uses anonymized data of individuals who cannot be identified. The results will be submitted to a peer-reviewed journal.

Introduction

Colorectal cancer is the third most common cancer worldwide, affecting over 1.3 million patients annually.¹ Approximately 50 per cent of these patients develop colorectal liver metastases (CRLM).² Although liver resection provides the best prospect of cure, only 10–30 per cent of patients with liver metastases are eligible for hepatic surgery.² Inoperability is mainly caused by patient morbidity in combination with number and size of hepatic tumours. For patients with tumours deemed unresectable, systemic chemotherapy can prolong survival, and allow potential future hepatic resection.^{3,4}

For decades, 5-fluorouracil was the sole active chemotherapeutic agent for CRLM. This has changed markedly in the new millennium: with the approval of irinotecan, oxaliplatin and humanized monoclonal antibodies, many more patients with initially unresectable CRLM became eligible for liver resection.^{5,6} Although response rates are convincing,³ administration of oxaliplatin and/or irinotecan-based chemotherapeutic agents has been associated with detrimental side-effects in the form of liver injury.⁷

Chemotherapy-associated liver injury (CALI) is often reported in patients with CRLM, and appears to be regimen-specific. For instance, oxaliplatin treatment is associated with sinusoidal obstruction syndrome (SOS),⁸ and increases the risk of nodular regenerative hyperplasia (NRH).⁹ Co-administration of bevacizumab with oxaliplatin, however, has been reported to decrease both the incidence and severity of SOS and NRH.^{8–10} Irinotecan-based regimens appear to be related to the development of steatohepatitis.^{7,11,12} Importantly, because patients commonly receive several chemotherapeutic agents to offer optimal benefit in downsizing tumours, it is difficult to identify the specific agents responsible for injury of the hepatic parenchyma.

Whereas certain studies claim an evident negative correlation between CALI and postoperative outcome (postoperative morbidity, mortality, tumour response and/or long-term survival),^{13–15} others could not reproduce this.^{14–21} Therefore, it remains unclear whether CALI influences postoperative morbidity and mortality.

Objectives

The aim of this study is to distinguish whether sinusoidal dilatation (SD), steatosis and steatohepatitis are risk factors for increased morbidity and mortality rates after partial hepatectomy by performing a meta-analysis of individual participant data based on a systematic literature review.

Methods

We developed the methods according to the PRISMA guidelines for conducting systematic reviews and meta-analyses²².

Literature search

The strategy for the literature search was developed within our systematic review group. J.Z. and K.v.M. will perform electronic searches in the databases of MEDLINE (PubMed) and Embase with the filters of publication dates (1 January 2004 to 31 December 2013). The publication date was determined to start from 2004 because the widely used criteria for scoring SD, steatosis and steatohepatitis were developed in 2004²³ and 2005.²⁴ We will not use language filters or any other filters to restrict the initial search. The search matrix includes four categories: liver resection, chemotherapy, tumour type and outcome. For the purpose of performing a more comprehensive search, we will not include the category of liver injury type (SD, steatosis, or steatohepatitis). *Table S3.1* lists the full search strategy.

Table S3.1 Search strategy

1st category	2nd category 2	3rd category	4th category	Filters
Liver resection	Chemotherapy	Tumour type	Outcomes	
Liver resection	Chemotherap* OR	Colorectal neoplasm	Morbidity OR	Dates: 01.01.2004-
OR Liver transection	Oxaliplatin OR	OR Colorectal cancer	Mortality OR Survival	31.12.2013
OR Hepatic	Irinotecan OR	OR Colorectal	OR Dead OR	Species: human
transection OR	FOLFOX OR	metastas* OR	Outcome OR	Age: >18 years
Hepatectomy	FOLFIRI OR	Colon cancer Liver	Recurrence OR Liver	
AND	CAPOX OR 5FU OR	metastas*	failure OR	
	Fluorouracil	AND	Regeneration OR	
	AND		Hepatic insufficiency	

Asterisks indicate blurred searching in case of synonymous words

Search protocol in PubMed and Embase

(((((liver resection) OR liver transection) OR hepatic transection) OR hepatectomy) AND (((((((chemotherap*) OR oxaliplatin) OR irinotecan) OR folfox*) OR folfiri) OR capox) OR 5fu) AND (((((((((((morbidity) OR mortality) OR dead) OR survival) OR outcome) OR risk) OR recurrence) OR regeneration) OR liver failure) OR hepatic failure) OR hepatotoxicity) OR bleeding) OR hemorrhage) OR infection) OR hepatopathy) OR prognosis) AND (((colorectal neoplasm*) OR colorectal cancer) OR colorectal metastas*) OR colon cancer)

Filters

- Publication dates: 1 January 2004 to 31 December 2013
- Species: humans
- Ages: adults (>18 years)

Checking of reference lists

Two authors (J.Z. and K.v.M.) will manually check the reference lists of included studies for additional potential citations. Moreover, an exploration of a personal library that was established because of previous research on this topic will be performed.²⁵

Identification and selection of studies

Studies identified by electronic searches and manual checking of reference lists will be imported into Endnote. The eligibility process will be conducted in two separate stages according to the inclusion criteria in *Table S3.2*.

1. Two authors (J.Z. and K.v.M.) will independently screen titles and abstracts of all non-duplicated papers and will exclude those not pertinent. A final list will be agreed with discrepancies resolved by consensus between the two authors. When consensus is not reached, a third author (S.O.D., S.R. or F.S.) will act as arbitrator. If any doubt about inclusion is dispelled, the article will proceed to the next stage.
2. The full-text version of the articles passing screening step 1 will be retrieved and assessed for eligibility by two authors (J.Z. and K.v.M.), independently. Discrepancies will be resolved by consensus between the two authors and, if needed, a third author (S.O.D., S.R. or F.S.) will act as arbitrator.

Table S3.2 Inclusion criteria

Articles published from January 2004 onwards
Study type: RCT, cohort study, case–control study, correlational study, case series and cross-sectional study.
Studies of case reports, comments, editorials, or reviews will be excluded
Population: adult patients (>18 years) who underwent liver resection for colorectal liver metastases
Outcome: studies with description of postoperative short-term overall morbidity, liver surgery-specific complications, postoperative liver failure, or mortality (≤ 90 days or in-hospital)
Pathological assessment: the severity of SOS was classified according to the criteria of Rubbia-Brandt <i>et al.</i> ²³
The severity of steatosis and steatohepatitis were classified according to the criteria of Kleiner <i>et al.</i> ²⁴

Quality assessment

Studies that meet the inclusion criteria will proceed to risk-of-bias assessment. As this is a systematic review for prognosis studies, we will adapt the QUIPS tool^{26,27} to assess the

quality of the final included studies. The QUIPS tool includes six bias domains: participation, attrition, prognostic factor measurement, confounding measurement and account, outcome measurement, and analysis and reporting. For each of the six domains, responses to the prompting items will be taken together to inform the judgement of bias risk. Judgements will be made with consensus among two authors (J.Z. and K.v.M.). Any discrepancies will be resolved by consensus between the two authors. If this is not possible, another author (S.O.D., S.R. or F.S.) will make a judgement on the quality and act as an arbitrator.

Data handling

Corresponding authors from studies that fulfilled the inclusion criteria will be contacted by e-mail for collaboration and sharing of published raw data. Once the author agrees on sharing the data, a preconstructed Excel™ file will be provided for the author to fill in the raw data. Each author will be asked to sign a specific data transfer agreements form, which assures careful handling of their data.

Statistical analysis

Shared raw data from the author will be pooled in SPSS® for Microsoft® Windows® (version 20.0). Patient characteristics will be compared using independent-samples *t* test for numerical variables, and Pearson Chi-square test or Fisher's exact test, where appropriate, for categorical variables. The influence of preoperative chemotherapeutic agents on liver injury, and subsequently the effect of the liver injury on short-term postoperative outcome, will be analysed by applying one-step binary logistic regression models. This approach is considered most optimal because some postoperative outcomes (such as liver failure, intraabdominal abscess or mortality) are usually small probability events, and the one-step approach for pooled data allows the exact binomial distribution to be used and does not require continuity corrections when no events occur.^{28,29}

As for missing values, we will consult a statistician and perform multiple imputations if necessary. The number of imputations will depend on the percentage of missing values. Once multiple imputations are performed, subsequent sensitivity analyses comparing the analyses of complete cases *versus* multiple imputative cases will be conducted. Clustering of patients from different studies will be integrated as a separate co-variable ('database source') and included in binary logistic regression models in every analysis.²⁸ A single variable together with database source will create the univariable model. A variable with $p \leq 0.200$ in univariable analysis will be included for multivariable analysis. In addition, database source and known influencing co-variables (either well described in

the literature or based on careful discussion and consensus between the authors) will be forced into the multivariable model. Unadjusted and adjusted odds ratios with 95% confidence intervals will be calculated. $p \leq 0.050$ will be considered significant for all analyses.

Data reporting

Data will be reported according to the PRISM and MOOSE guidelines for systematic reviews. The manuscript will be reviewed and approved by all authors, and published in a peer-reviewed journal.

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Appendix 3.2 Variables in multiple imputations

Patient cluster	General	Chemotherapy	Liver injury	Surgical factors	Outcome
Database source	Sex, age, BMI, co-morbidity	5-Fluorouracil, capecitabine, irinotecan, bevacizumab, cetuximab, oxaliplatin	Sinusoidal dilatation, steatosis, steatohepatitis	Resection type, PVE, pringle manoeuvre, transfusion of packed RBCs	Hospital stay, overall morbidity, complications (minor/major), liver surgery-specific complication, mortality, liver failure

PVE, portal vein embolization; RBC, red blood cell

Appendix 3.3 Flow chart summarizing final inclusion process

Appendix 3.4 Quality in Prognostic Studies detailed risk-of-bias assessment of individual included studies

	Gómez-Ramírez <i>et al.</i> ³⁹	Nam <i>et al.</i> ⁴⁰	Pessaux <i>et al.</i> ⁴¹	Pilgrim <i>et al.</i> ¹⁶	Soubrane <i>et al.</i> ⁴²	Takamoto <i>et al.</i> ⁴³	van der Pool <i>et al.</i> ⁴⁴	Viganò <i>et al.</i> ¹⁷
1. Study participation								
Source of target population	✓	✓	✓	✓	✓	✓	✓	✓
Method used to identify population	✓	✓	✓	✓	✓	✓	✓	✓
Recruitment period	✓	✓	✓	✓	✓	✓	✓	✓
Place of recruitment	✓	✓	✓	✓	✓	✓	✓	✓
Inclusion and exclusion criteria	✓	✓	✓	✓	✓	✓	✓	✓
Adequate study participation	✓	✓	✓	✓	✓	✓	✓	✓
Baseline characteristics	✓	✓	✓	✓	✓	✓	✓	✓
Summary study participation	low	low	low	low	low	low	low	low
2. Study attrition								
Summary study attrition	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
3. PF measurement								
Definition of the PF	✓	✓	✓	✓	✓	✓	✓	✓
Valid and reliable measurement of PF	✓	✓	✓	✓	✓	✓	✓	✓
Reporting continuous variables	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Method and setting of PF measurement	✓	✓	✓	✓	✓	✓	✓	✓
Proportion of data on PF available for analysis	✓	✓	✓	✓	✓	✓	✓	✓
Method used for missing data	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
PF measurement summary	low	low	low	Low	low	low	low	low

	Gómez-Ramírez et al. ³⁹	Nam et al. ⁴⁰	Pessaux et al. ⁴¹	Pilgrim et al. ¹⁶	Soubrane et al. ⁴²	Takamoto et al. ⁴³	van der Pool et al. ⁴⁴	Viganò et al. ¹⁷
4. Outcome measurement								
Definition of the outcome	✓	✓	✓	✓	✓	✓	✓	✓
Valid and reliable measurement of outcome	✓	✓	✓	✓	✓	✓	✓	✓
Method and setting of outcome measurement	✓	✓	✓	✓	✓	✓	✓	✓
Outcome measurement summary	low	low	low	mod	low	mod	mod	low
5. Study confounding								
Important confounders measured	✓	✓	✓	✓	✓	✓	✓	✓
Definition of the confounding factor	✓	✓	✓	✓	✓	✓	✓	✓
Valid and reliable measurement of confounders	✓	✓	✓	✓	✓	✓	✓	✓
Method and setting of confounding measurement	✓	✓	✓	✓	✓	✓	✓	✓
Method used for missing data	✓	?	✓	?	✓	✓	✓	✓
Appropriate accounting for confounding in study design	✓	✓	✓	✓	✓	✓	✓	✓
Appropriate accounting for confounding in analysis	✗	✗	✗	✗	✗	✗	✗	✗
Study confounding summary	mod	mod	mod	low	mod	mod	mod	mod
6. Statistical analysis and reporting								
Presentation of analytical strategy	✓	✓	✓	✓	✓	✓	✓	✓
Appropriate model building	✓	✓	✗	✓	✓	✓	✓	✓
Adequate statistical model	✓	✓	✗	✓	✓	✓	✓	✓
Reporting of results	✓	✓	✓	✓	✓	✓	✓	✓
Statistical analysis and presentation summary	mod	low	high	low	low	mod	low	low
Overall assessment	low	low	high	low	low	mod	low	low

✓, Yes; ✓~, partial; ✗, no; ?, unsure; mod, moderate; n.a., not applicable. PF, prognostic factor.

Appendix 3.5 Relationship between non-alcoholic fatty liver disease activity score and lobular inflammation

NAS	Lobular inflammation (foci per 200 × field)				Total no. of patients
	0	< 2	2–4	> 4	
0	100	0	0	0	100
1	59	130	0	0	189
2	24	100	24	0	148
3	6	46	39	11	102
4	3	26	32	7	68
5	0	5	7	4	16
6	0	1	4	4	9
7	0	0	2	1	3
Total no. of patients	192	308	108	27	635

NAS, non-alcoholic fatty liver disease activity score

Appendix 3.6 Influence of liver injury on short-term postoperative outcomes

	Overall morbidity			Liver surgery-specific complications						Major morbidity (DC III-V)		
	Univariable analysis		P	Multivariable analysis		P	Univariable analysis		P	Multivariable analysis		P
	Odds ratio	Odds ratio		Odds ratio	Odds ratio		Odds ratio	Odds ratio		Odds ratio	Odds ratio	
Sex (F)	0.63 (0.47, 0.85)	0.61 (0.45, 0.83)	0.002	0.002	0.72 (0.51, 1.02)	0.066	0.65 (0.45, 0.94)	1.01 (0.65, 1.57)	0.980			
Age (years)	1.01 (1.00, 1.02)	1.01 (1.00, 1.02)	0.122	0.164	0.99 (0.98, 1.01)	0.450		1.02 (0.99, 1.04)	0.179	1.02 (0.99, 1.04)		0.137
BMI (kg/m ²)	0.97 (0.93, 1.01)	0.97 (0.93, 1.01)	0.086	0.106	0.92 (0.88, 0.96)	<0.001	0.93 (0.89, 0.98)	0.99 (0.93, 1.05)	0.654			
Co-morbidity	1.02 (0.57, 1.83)		0.950		0.76 (0.30, 1.88)	0.537		1.01 (0.63, 1.61)	0.972			
PVE	1.85 (1.13, 3.05)	1.31 (0.74, 2.32)	0.015	0.358	3.84 (2.34, 6.31)	<0.001	2.64 (1.46, 4.78)	2.01 (1.11, 3.64)	0.021	1.50 (0.76, 2.96)		0.246
Preoperative CTx	0.90 (0.61, 1.33)		0.594		0.98 (0.62, 1.54)	0.924		1.43 (0.73, 2.82)	0.298			
Resection type (major)	2.02 (1.50, 2.72)	1.72 (1.24, 2.40)	<0.001	0.001	2.42 (1.69, 3.48)	<0.001	1.72 (1.14, 2.58)	1.72 (1.03, 2.85)	0.010	1.21 (0.67, 2.22)		0.526
Pringle manoeuvre	1.23 (0.91, 1.65)	0.97 (0.70, 1.34)	0.185	0.834	1.38 (0.96, 1.97)	0.079	0.93 (0.61, 1.39)	1.38 (0.86, 2.19)	0.706	1.12 (0.67, 1.88)		0.667
Blood transfusion	2.98 (2.01, 4.41)	2.53 (1.68, 3.80)	<0.001	<0.001	2.17 (1.45, 3.26)	<0.001	1.73 (1.10, 2.70)	3.28 (2.01, 5.35)	0.017	2.77 (1.66, 4.61)		<0.001
Severe SD	1.52 (1.09, 2.13)	1.25 (0.86, 1.80)	0.014	0.239	1.87 (1.29, 2.71)	0.001	1.28 (0.83, 1.98)	2.09 (1.30, 3.38)	0.268	1.73 (1.02, 2.95)		0.043
Severe steatosis	1.03 (0.69, 1.54)		0.872		0.52 (0.30, 0.90)	0.020	0.52 (0.27, 1.00)	1.07 (0.55, 2.09)	0.049			
Steatohepatitis	1.67 (1.11, 2.51)	1.58 (0.99, 2.52)	0.014	0.057	1.61 (0.75, 3.46)	0.217	2.08 (1.18, 3.66)	1.85 (0.94, 3.63)	0.012*	1.67 (0.85, 3.30)		0.139

Values in parentheses are 95 per cent confidence intervals. DC, Dindo-Clavien grade; PVE, portal venous embolization; CTx, chemotherapy; SD, sinusoidal dilatation. *Multiple imputations were used for univariable and multivariable analyses. *As steatohepatitis was frequently reported as a risk factor for postoperative morbidity and had a P value close to the upper limit for inclusion (0.200), it was forced into multivariable analysis.

Appendix 3.7 Factors related to liver injury

	Severe SD				Severe steatosis				Steatohepatitis			
	Univariable analysis		Multivariable analysis		Univariable analysis		Multivariable analysis		Univariable analysis		Multivariable analysis	
	Odds ratio	P	Odds ratio	P	Odds ratio	P	Odds ratio	P	Odds ratio	P	Odds ratio	P
Sex (F)	1.14 (0.81, 1.60)	0.451			0.93 (0.62, 1.39)	0.716			0.98 (0.61, 1.57)	0.932		
Age (years)	0.99 (0.98, 1.01)	0.354			1.00 (0.98, 1.02)	0.735			1.00 (0.98, 1.02)	0.862		
BMI (kg/m ²)	0.94 (0.90, 0.98)	0.004	0.97 (0.92, 1.01)	0.156	1.17 (1.11, 1.22)	< 0.001	1.15 (1.08, 1.21)	< 0.001	1.07 (0.99, 1.15)	0.098	1.00 (0.93, 1.07)	0.997
PVE	2.36 (1.44, 3.88)	0.001	1.62 (0.95, 2.75)	0.074	0.33 (0.12, 0.92)	0.033	0.29 (0.08, 1.00)	0.050	1.32 (0.12, 15.01)	0.821		
Co-morbidity	0.77 (0.51, 1.15)	0.204			1.81 (1.19, 2.76)	0.006	1.44 (0.69, 2.99)	0.324	1.06 (0.50, 2.21)	0.884		
5-FU	1.98 (1.34, 2.91)	0.001	1.20 (0.71, 2.04)	0.491	1.08 (0.69, 1.68)	0.747			1.79 (0.44, 7.22)	0.403		
Capecitabine	0.67 (0.42, 1.06)	0.089	0.69 (0.38, 1.26)	0.231	1.52 (0.94, 2.45)	0.087	1.53 (0.75, 3.12)	0.246	1.22 (0.42, 3.50)	0.712		
Oxaliplatin	2.89 (1.93, 4.31)	< 0.001	2.74 (1.67, 4.49)	< 0.001	0.99 (0.64, 1.51)	0.943			1.94 (0.44, 8.62)	0.372		
Bevacizumab	0.71 (0.50, 1.13)	0.145	0.72 (0.43, 1.20)	0.207	1.39 (0.86, 2.24)	0.177	1.09 (0.59, 2.01)	0.787	1.37 (0.80, 2.36)	0.252		
Cetuximab	1.95 (1.01, 3.75)	0.045	1.67 (0.85, 3.29)	0.134	0.71 (0.33, 1.54)	0.391			0.72 (0.28, 1.90)	0.512		
Irinotecan	0.67 (0.39, 1.16)	0.153	0.73 (0.40, 1.34)	0.315	1.70 (0.97, 2.99)	0.065	1.85 (0.84, 4.05)	0.126	1.10 (0.42, 2.89)	0.852	0.75 (0.21, 2.71)	0.654*
Severe steatosis	0.35 (0.19, 0.64)	0.001	0.44 (0.24, 0.83)	0.011	—	—	—	—	14.52 (6.07, 34.71)	< 0.001	15.09 (6.25, 36.45)	< 0.001
Severe SD	—	—	—	—	0.35 (0.19, 0.64)	0.001	0.36 (0.15, 0.88)	0.025	1.06 (0.20, 5.45)	0.947	—	—
Steatohepatitis	1.08 (0.21, 5.67)	0.928			13.76 (5.30, 35.70)	< 0.001	15.46 (7.53, 31.76)	< 0.001	—	—	—	—

Values in parentheses are 95 per cent confidence intervals. SD, sinusoidal dilatation; PVE, portal venous embolization; 5-FU, 5-fluorouracil. Multiple imputations were used for univariable and multivariable analyses. *As irinotecan was frequently reported as a risk factor for postoperative morbidity and had a *P* value close to the upper limit for inclusion (0.200), it was forced into multivariable analysis.

Chapter 4

Impact of chemotherapy-associated liver injury on postoperative infectious complications after partial hepatectomy for colorectal liver metastases

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Abstract

Introduction

Although postoperative infectious complications and chemotherapy-associated liver injury (CALI) are both major concerns for liver surgery in patients with colorectal liver metastases (CRLM), little is known regarding their relation. The aim of the current study was to investigate potential associations between CALI, postoperative infectious complications, and survival after partial hepatectomy.

Methods

Patients who underwent partial hepatectomy for CRLM between 2008 and 2014 were included. CALI was assessed histopathologically, focusing on steatosis, steatohepatitis, nodular regeneration, and sinusoidal dilatation. Infectious complications were prospectively monitored daily. Multivariable regression was performed, and results were depicted as odds ratio (OR) or hazard ratio (HR) with 95% confidence interval (CI).

Results

265 patients were included with a median period of follow-up of 45 and 54 months for recurrence-free and overall survival, respectively. Grade 2-3 sinusoidal dilatation was found in 66 (24.9%) patients, grade 2-3 steatosis in 30 (11.3%) patients, and steatohepatitis in one (0.6%) patient. With hardly observed liver fibrosis, nodular regeneration was present in 41 (19.3%) of 212 patients with collagen staining available. In multivariable analysis, severe steatosis was related to increased surgical site infections (OR 3.55, 95% CI 1.39-9.07, $p=0.008$) and systemic sepsis (OR 6.73, 95% CI 1.34-33.73, $p=0.020$). Neither sinusoidal dilatation nor nodular regeneration was related to infectious complications. Presence of deep or organ/space surgical site infections were associated with decreased recurrence-free (HR 1.71, 95% CI 1.04-2.80, $p=0.035$), and overall survival (HR 2.04, 95% CI 1.31-3.19, $p=0.002$). None of the studied background liver abnormalities was related to survival.

Conclusions

Chemotherapy-associated hepatic steatosis was associated with increased infectious complications, which were, in turn, related to decreased recurrence-free and overall survival.

Introduction

Postoperative infectious complications occur often after partial hepatectomy for colorectal liver metastases.¹ The presence of infectious complications, particularly surgical site infections, remains a crucial clinical problem and is related to prolonged length of hospital stay,¹ increased healthcare cost,^{2,3} in-hospital mortality,⁴ and even decreased long-term survival.^{4,5} Although many advances have been made in infection control in the past decades, surgical site infections remain the second most frequent healthcare-related infection after respiratory tract infection.⁶

Neoadjuvant chemotherapy for patients with colorectal liver metastases is used to downstage the tumour and to facilitate future hepatic resection.^{7,8} However, systemic chemotherapy may also cause undesired liver parenchymal damage, referred to as chemotherapy-associated liver injury (CALI). For instance, the combination of 5-fluorouracil and leucovorin is related to the development of steatosis,⁹ irinotecan-based regimens are associated with increased occurrence of steatohepatitis,⁹⁻¹¹ and oxaliplatin-based treatments appear to be related to the development of sinusoidal obstruction syndrome¹² as well as nodular regeneration.¹³

The development of CALI has several, potentially serious adverse downstream effects. It has been shown by our group¹⁴ and subsequently by others¹⁵ that CALI is associated with poor tumour response to neoadjuvant chemotherapy. Moreover, CALI is related to increased surgical morbidity¹⁶⁻¹⁹ and mortality,²⁰ and may even reduce survival²¹ of patients with colorectal liver metastases undergoing partial hepatectomy. Unfortunately, our understanding of the impact of CALI on the incidence of postoperative infectious complications after partial hepatectomy is poor. Therefore, the primary aim of the present study was to explore whether CALI (i.e. sinusoidal dilatation, nodular regeneration, steatosis, and steatohepatitis) is associated with increased infectious complications, and, secondly, to investigate if these factors are associated with survival of patients with colorectal liver metastases who underwent partial hepatectomy.

Methods

Inclusion of patients

Consecutive patients who had undergone partial hepatectomy for colorectal liver metastases at Maastricht University Medical Centre, a tertiary referral hospital, between January 2008 and December 2013 were considered for this study if there was an

adequate histopathological assessment of non-tumour bearing liver tissue (i.e. presence of non-tumour-bearing liver at a distance of more than 2 cm from the tumour). Patients with cirrhosis were excluded from the study.

Definitions and data collection

Data regarding the postoperative infectious complications were prospectively monitored daily by an independent infection-control nurse (MR) based on the following definitions. Postoperative infectious complications were categorized as a combination of surgical site infections, remote site infections, and systemic sepsis developed within 30 days after surgery or during hospital stay. Surgical site infections were defined according to the guideline of the Centres for Disease Control and Prevention (CDC)'s recommendations, including superficial incisional, deep incisional, and intraabdominal organ/space infections.²² Severe surgical site infections were defined as the presence of either deep incisional or intraabdominal organ/space infections. Remote site infections were described as a condition in which fever and leukocytosis were present with bacteria in urine, sputum, and/or central venous catheter tips according to an experienced physician's judgment with consideration of microbiologic evidence when available.

Comorbidity was defined as any disease affecting the patient apart from colorectal liver metastases (i.e. diabetes mellitus, and pulmonary, renal, cardiovascular, and other diseases). Overall morbidity was defined as any complication occurring within 30 days after surgery or during hospital stay and was categorized according to Dindo et al.²³ Radical resection was defined as resected tumour lesions with a surgical margin of more than one millimeter. All patients were admitted one-day prior to liver surgery, and the length of hospital stay was defined as the duration between the date of admission and the date of discharge.

Pathology assessment of non-tumour-bearing liver

Sinusoidal dilatation and nodular regeneration were semi-quantitatively assessed by two experienced liver pathologists (AW and CV). Diagnosis of nodular regeneration was made only when confirming liver fibrosis was absent or minor. Assessment of nonalcoholic fatty liver disease was performed by another experienced liver pathologist (JV) independently. All pathologists were blinded to clinical information concerning the patients.

The resected liver tissue was fixed in formalin, embedded in paraffin, and stained with hematoxylin & eosin and reticulin. Sinusoidal dilatation was graded according to Rubbia-Brandt et al.²⁴ Nodular regeneration was graded according to the Wanless scoring system.²⁵ Steatosis was graded according to Kleiner et al.²⁶ According to the recently established SAF scoring system, a case presenting with at least grade 1 of each of the three features (i.e. steatosis, hepatocellular ballooning, and lobular inflammation) was classified as steatohepatitis.²⁷

Statistical analyses

All statistical analyses were performed using IBM SPSS Statistics for Windows (version 20.0), and all graphs were drawn using GraphPad Prism for Windows (Version 6.05). For multiple comparisons of numerical variables, patient characteristics were compared using One-way ANOVA and Tukey's multiple comparisons test. For categorical variables, the Pearson Chi-square test with continuity correction was applied, or the Fisher's exact test when any of the expected values was smaller than five. The relation between CALI and postoperative infectious complications was studied applying uni- and multivariable binary logistic regression models.

The association between CALI, infectious complications, and survival was analyzed using uni- and multivariable Cox regression models. To specify which type of postoperative morbidities were the key component related to survival, multivariable analysis was performed subsequently in three-steps: step one, overall morbidity vs. absent morbidity; step two, any infectious morbidity vs. non-infectious morbidity vs. absent morbidity; step three, severe surgical site infections vs. remote site infections vs. superficial surgical site infections or sepsis vs. non-infectious morbidity vs. absent morbidity.

Variables with p-value ≤ 0.10 in univariable analysis were included in the multivariable analysis. Odds ratios (OR) or hazard ratios (HR) with 95% confidence intervals (CI) were calculated. A p-value ≤ 0.05 was considered significant for all tests.

Results

A total of 265 consecutive patients were included (Table 4.1), of which 172 (64.9%) were male and 93 (35.1%) female, with a median age of 66 (inter quartile range 59-72) years.

Table 4.1 General characteristics

Factors	n=265
General characteristics	
Sex (male)	172 (64.9%)
Age (year)	66 (59-72)
Body mass index (kg/m ²)	26.0 (23.7-28.6)
Comorbidity	169 (63.8%)
Diabetes	22 (8.3%)
Cardiovascular	117 (44.2%)
Pulmonary	28 (10.6%)
Renal	8 (3.0%)
Others	71 (26.8%)
Portal venous embolization	12 (4.5%)
Primary colorectal tumour	
Rectal cancer	108 (40.8%)
Tumour stage 3-4 (n=260*)	220 (83.0%)
Lymph node with tumour cells (n=257*)	167 (63.0%)
Liver metastases	
Synchronous metastases (<12 months)	183 (69.1%)
Number of metastases	2 (1-3)
Diameter of largest metastases	2.5 (1.5-4.0)
Chemotherapy details	
Preoperative chemotherapy	184 (69.4%)
5-fluorouracil	6 (2.3%)
Oxaliplatin	167 (61.9%)
Capecitabine	170 (64.2%)
Irinotecan	9 (3.4%)
Bevacizumab	114 (43.0%)
Surgical details	
Major hepatectomy (≥3 Couinaud segments)	119 (44.9%)
Surgical duration in hour (n=263*)	3.4 (2.5-4.5)
Pringle manoeuvre (n=264*)	67 (25.3%)
Laparoscopic surgery	24 (9.1%)
Perioperative blood transfusion	48 (18.1%)
Radical resection	184 (69.4%)
Postoperative characteristics	
Overall morbidity	125 (47.2%)
Infectious complications	82 (30.9%)
Surgical site infections	63 (23.8%)
Superficial wound infection	33 (12.5%)
Deep wound infection	9 (3.4%)
Intraabdominal abscess	16 (6.0%)
Liver abscess	23 (8.7%)
Remote site infections	41 (15.5%)
Urinary tract infection	21 (7.9%)
Respiratory infection	26 (9.8%)
Central venous catheter infection	6 (2.3%)
Sepsis	20 (7.5%)
Ninety-day mortality	11 (4.2%)
Length of hospital stay (day)	8 (6-11)

Numeric variables are presented as median with interquartile range; *Due to missing data, indicated number of patients was below 265.

Preoperative comorbidity was present in 169 (63.8%) patients. Twelve (4.5%) patients had undergone unilateral portal venous embolization before liver surgery. 148 (55.8%) patients developed more than one liver metastasis. 184 (69.4%) patients received preoperative chemotherapy. Capecitabine in combination with oxaliplatin (CAPOX) was the most commonly used regimen, and was administered to 167 (90.8%) patients. Bevacizumab was co-administered to 114 (62.0%) patients. Postoperative surgical site infections were present in 63 (23.8%) patients, remote site infections were found in 41 (15.5%) patients, and systemic sepsis was observed in 20 (7.5%) patients. The median length of hospital stay was 8 (interquartile range 6-11) days, and 247 (93.8%) patients were discharged within 30 days after admission.

Table 4.2 summarizes the pathology details of chemotherapy-associated liver injury. Of the 148 (55.8%) patients who presented with sinusoidal dilatation, mild lesions (grade 1) were found in 82 (30.9%) subjects, and severe lesions (grade 2 or grade 3) were observed in 66 (24.9%) patients. Mild steatosis (grade 1) was present in 21 (7.9%) patients, and severe steatosis (grade 2 and grade 3) was found in 30 (11.3%) patients. Collagen staining, which was required to assess nodular regeneration, was available from 212 patients, and revealed nodular regeneration in 41 (15.5%) of them; severe nodular regeneration (grade 2 or grade 3) was observed in 20 (9.4%) patients. Steatohepatitis was found in one (0.4%) patient, who had grade 2 steatosis (40% steatotic hepatocytes), grade 1 lobular inflammation, and grade 2 hepatocellular ballooning, but absent sinusoidal dilatation.

Table 4.2 Pathology characteristics.

Pathology details	n=265
Sinusoidal dilatation	
Absent	117 (44.2%)
Grade 1	82 (30.9%)
Grade 2	43 (16.2%)
Grade 3	23 (8.7%)
Nodular regeneration (n=212*)	
Absent	171 (80.7%)
Grade 1	21 (7.9%)
Grade 2	13 (4.9%)
Grade 3	7 (2.6%)
Steatosis	
Absent	109 (41.1%)
Grade 1	126 (47.5%)
Grade 2	22 (8.3%)
Grade 3	8 (3.0%)
Steatohepatitis	1 (0.4%)

*Due to unavailable collagen staining, indicated number of patients was below 265.

The presence of severe sinusoidal dilatation was higher in patients who presented with nodular regeneration than in those without these lesions (n=212, 28/41 vs. 31/171, $p<0.001$). In contrast, the incidence of severe sinusoidal dilatation in patients with severe steatosis was lower than that of non-severe lesions (1/30 vs. 65/235, $p=0.002$). Subgroup multivariable analysis (Supplemental Table S4.1) showed that both grade 1 (OR 0.32, 95% CI 0.12-0.86, $p=0.024$) and grade 2-3 sinusoidal dilatation (OR 0.05, 95% CI 0.01-0.43, $p=0.006$) were inversely related to the occurrence of severe steatosis. Both irinotecan-based treatment (OR 9.66, 95% CI 1.42-65.49, $p=0.020$) and body mass index (OR 1.27, 95% CI 1.13-1.42, $p<0.001$) were independently associated with increased severe steatosis.

Multivariable analysis showed that perioperative blood transfusion (OR 2.14, 95% CI 1.09-4.22, $p=0.027$) was the sole factor significantly related to increased infectious complications. Surgical duration (OR 1.14, 95% CI 0.98-1.33, $p=0.098$) and severe steatosis (OR 2.33, 95% CI 1.97-5.59, $p=0.059$) tended to be associated with increased infectious complications. To clarify which type of infectious complications (e.g. surgical site infections, remote site infections, and systemic sepsis) was associated with background liver abnormalities, further multivariable analyses were performed (Figure 4.1, detailed data are summarized in supplemental Table S4.2).

Figure 4.1 Factors related to postoperative infectious complications. Data are depicted as odds ratios (symbols) with 95% confidence intervals (bars) based on results of multivariable analysis, variable with $p\leq 0.10$ in univariable analysis were induced in the multivariable analysis, one asterisk indicates $p\text{-value}\leq 0.05$, two asterisks indicate $p\text{-value}\leq 0.01$.

Severe steatosis was associated with increased incidence of surgical site infections (OR 3.55, 95% CI 1.39-9.07, $p=0.008$). Both grade 1 (OR 4.38, 95% CI 1.18-16.21) and grade 2-3 steatosis (OR 6.73, 95% CI 1.34-33.73, $p=0.020$) were related to systemic sepsis. Sinusoidal dilatation and nodular regeneration were not related to any type of infectious complications. The presence of comorbidity was the sole factor significantly related to

remote site infections (OR 2.59, 95% CI 1.15-5.84, $p=0.022$). To investigate whether steatosis was related to superficial and/or severe surgical site infections, a subgroup analysis was performed (supplemental Table S4.3). It was shown that severe steatosis was only related to increased superficial surgical site infections (OR 4.50, 95% CI 1.43-14.10, $p=0.010$), but not to severe surgical site infections (OR 1.14, 95% CI 0.34-3.78, $p=0.835$). Both major hepatectomy (OR 2.31, 95% CI 1.10-4.84, $p=0.027$) and surgical duration (OR 1.20, 95% CI 1.01-1.42, $p=0.037$) were associated with increased severe surgical site infections.

The relationship between potential prognostic factors and recurrence-free survival is summarized in Figure 4.2 (detailed data are summarized in supplemental Table S4.4). The median period of follow-up for recurrence-free survival was 45 months. None of the studied background liver abnormalities was related to recurrence-free survival. In multivariable analysis, the presence of severe surgical site infections was the sole factor related to a 71 percent increased hazard of tumour recurrence (HR 1.71, 95% CI 1.04-2.80, $p=0.035$). Moreover, remote site infections tended to be associated with recurrence-free survival (HR 1.68, 95% CI 0.91-3.08, $p=0.096$). Both radical resection (HR 0.54, 95% CI 0.37-0.79, $p<0.001$) and body mass index (HR 0.95, 95% CI 0.91-0.99, $p=0.015$) were associated with decreased risk of tumour recurrence.

Figure 4.2 Prognostic factors related to recurrence-free survival. Data are depicted as hazard ratios (symbols) with 95% confidence intervals (bars) based on results of multivariable analysis; one asterisk indicates p -value ≤ 0.05 , two asterisks indicate p -value ≤ 0.01 , and three asterisks indicate p -value ≤ 0.001 ; SSIs, surgical site infections; RSIs, remote site infections.

Figure 4.3 summarizes the association between potential prognostic factors and overall survival (detailed data are summarized in supplemental Table S4.5). Median period of follow-up for overall survival was 54 months. Again, none of the studied background liver abnormalities was associated with overall survival. Severe surgical site infections were related to a 104 percent increased hazard of mortality (HR 2.04, 95% CI 1.31-3.19, $p=0.002$). Remote site infections (HR 1.22, 95% CI 0.71-2.12, $p=0.474$), superficial surgical site infections or sepsis (HR 1.35, 95% CI 0.69-2.66, $p=0.379$), and non-infectious complications (HR 0.83, 95% CI 0.50-1.37, $p=0.466$) were not associated with overall survival. Age (HR 1.02, 95% CI 1.00-1.04, $p=0.025$), synchronous liver metastases (HR 1.51, 95% CI 1.03-2.20, $p=0.033$), and the number of liver metastases (HR 1.11, 95% CI 1.03-1.19, $p=0.005$) were related to decreased overall survival. Conversely, radical resection (HR 0.52, 95% CI 0.37-0.74, $p<0.001$) and major hepatectomy (HR 0.64, 95% CI 0.44-0.93, $p=0.018$) were associated with increased overall survival.

Figure 4.3 Prognostic factors related to overall survival. Data are depicted as hazard ratios (symbols) with 95% confidence intervals (bars) based on results of multivariable analysis; one asterisk indicates p -value ≤ 0.05 , two asterisks indicate p -value ≤ 0.01 , and three asterisks indicate p -value ≤ 0.001 ; SSIs, surgical site infections; RSIs, remote site infections.

Next, we also explored the association between postoperative morbidity, 90-day mortality, and length of hospital stay (Figure 4.4). 90-day mortality occurred more often in patients with severe surgical site infections than in those without (6/39 vs. 3/226, $p<0.001$). Of the occurred six deaths in the group of patients with severe surgical site infections, four patients had liver abscesses, accompanied with one deep wound infection and one intra-abdominal abscess; one patient had only deep wound infection, and another one had intra-abdominal abscess. The mean length of hospital stay for patients with severe surgical site infections was 26.3 ± 4.3 days. This was considerably longer than length of stay of patients without morbidity (mean difference 19.3 days, 95% CI 13.1-25.4, $p<0.001$), with non-infectious complications (mean difference 14.3 days, 95% CI 6.8-21.8, $p<0.001$), with superficial surgical site infections/sepsis (mean difference 14.0 days, 95% CI 4.9-23.2, $p<0.001$), and with remote site infections (mean difference 13.6 days, 95% CI 4.6-22.6, $p<0.001$).

Figure 4.4 Postoperative morbidity and length of hospital stay. One way ANOVA, $p<0.001$; Turkey's multiple comparisons test: *** indicates p -value <0.001 ; Error bars represent means with standard error of means; RSIs, remote site infections; SSIs, surgical site infections.

Discussion

In the present study, surgical site infections and systemic sepsis after partial hepatectomy for colorectal liver metastases were found to occur more often in patients with hepatic steatosis, whereas neither sinusoidal dilatation nor nodular regeneration were associated with these infectious complications. Steatosis was specifically related to increased superficial surgical site infections. The presence of severe surgical site infections was associated with decreased long-term recurrence-free survival, and overall survival. None of the studied background liver abnormalities was related to survival. Contrasting to the observed inverse relation between severe steatosis and sinusoidal dilatation, severe sinusoidal dilatation was positively related to the occurrence of nodular regeneration. Irinotecan-based treatment and body mass index were also related to increased severe steatosis.

The fact that severe steatosis was independently associated with the occurrence of surgical site infections is in line with a previous report,²⁸ and might be related to the body mass index of patients. As confirmed in the current cohort, an increased body mass index is a risk factor for developing steatosis.²⁹ A higher BMI may increase the extent of subcutaneous fat accumulation, which may cause increased tension on surgical sutures, leading to slower wound healing and increased susceptibility to infection. Furthermore, a change in gut microbiota composition seen in obese individuals are known to be associated with hepatic insulin resistance, an important cause of hepatic steatosis,³⁰ and with bacterial translocation,³¹ which may further contribute to increased susceptibility to systemic sepsis. Hepatic steatosis may additionally diminish the ability of Kupffer cells to clear translocated bacteria.³² In addition, excessive adipose tissue accumulation in obese individuals promotes dysregulated expression of adipokines like leptin, visfatin, adiponin, and angiopoietin-like protein 2 that affect the immune system, potentially increasing susceptibility to infections.³³

Next to the link between hepatic steatosis and surgical site infections, this study showed that infectious complications, rather than non-infectious events, were related to decreased survival after partial hepatectomy for colorectal liver metastases. In particular, the presence of severe surgical site infections was related to a significantly increased risk of tumour recurrence and long-term mortality, consistent with a previous report⁵. Interestingly, it has been shown by Akhter et al. that surgical site infections occur more often in patients with immune suppression.³⁴ In accordance with this, enhancing the immune system may suppress tumour progression,³⁵ enhance tumour response, and prolong long-term survival.³⁶ In essence, the presence of severe surgical

site infections may mirror a relatively weaker innate or adaptive immunity. Tumour suppression may therefore be weakened, eventually resulting in more frequent tumour recurrence and decreased long-term overall survival.

Although Tamandl et al. showed a correlation between chemotherapy-associated sinusoidal dilatation and long-term survival after partial hepatectomy for colorectal liver metastases,²¹ neither sinusoidal dilatation nor any other type of liver injury was related to survival in the current study. In the previous cohort,²¹ only patients with preoperative neoadjuvant chemotherapy were included, minimizing the influence of factors other than preoperative chemotherapeutic treatment for developing background liver abnormalities. More importantly, the effect of neoadjuvant chemotherapy on the tumour, expressed as tumour response which is shown to be positively related to survival,¹⁵ whereas negatively related to the presence of chemotherapy-associated liver injury,^{14,15} may be taken into account potentially. Therefore, future study is needed to determine whether CALI itself or an impaired tumour response as a result of CALI are associated with long-term recurrence-free survival and overall survival after partial hepatectomy for colorectal liver metastases. Because postoperative infectious complications were important prognostic factors for survival in the present study, such factors should be included as confounders in future studies.

Remarkably, in the present study, only one patient was diagnosed with steatohepatitis. This could be explained by the fact that few patients were treated with irinotecan, a known risk factor for steatohepatitis.⁹⁻¹¹ Alternatively, it could also be due to the fact that steatohepatitis was diagnosed applying the novel SAF scoring system which is more strict as compared to the previously used NAS system.²⁶

Because chemotherapy was frequently administered at local referral hospitals, data on the interval between cessation of preoperative chemotherapy and partial hepatectomy, as well as the number of administered cycles, were not available for the majority of studied patients. Therefore, the potential effect of these factors on postoperative infectious complications and survival could not be studied. However, the majority of patients treated with chemotherapy received the same regimen (capecitabine in combination with oxaliplatin). Therefore, the influence of these factors on infectious complications and survival was presumably minimal. Finally, whereas the current study featured a single-centre, retrospective study design, it must be highlighted that data regarding postoperative infectious complications were prospectively collected daily by an experienced independent infection-control nurse.

In conclusion, this study tried to explore the relationship between two important issues in hepatic surgery (i.e. chemotherapy-associated liver injury and postoperative infectious complications), and their association with survival after partial hepatectomy for colorectal liver metastases. We demonstrated that postoperative surgical site infections and systemic sepsis occurred more often in patients with hepatic steatosis. In particular, the presence of steatosis was related to increased superficial surgical site infections, instead of severe surgical site infections. Severe surgical site infections were associated with increased 90-day mortality and length of hospital stay, and decreased recurrence-free and overall survival. It seems thus reasonable to advise clinicians to adopt preventive strategies concerning infection control when hepatic steatosis is observed through preoperative ultrasound or radiological images, or even if a “yellow liver” is seen during surgery.

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Supplemental tables

Table S4.1 Factors related to hepatic steatosis.

Factors	Severe steatosis			
	univariable		multivariable	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Sex (male)	0.93 (0.42-2.04)	0.848	-	-
Age (year)	0.99 (0.96-1.03)	0.706	-	-
Body mass index (kg/m ²)	1.25 (1.12-1.39)	<0.001	1.27 (1.13-1.42)	<0.001
Comorbidity	0.71 (0.33-1.54)	0.391	-	-
Portal venous embolization	0.70 (0.09-5.64)	0.739	-	-
5-fluorouracil	1.59 (0.18-14.05)	0.679	-	-
Oxaliplatin	1.26 (0.57-2.82)	0.568	-	-
Capecitabine	1.35 (0.59-3.07)	0.479	-	-
Irinotecan [#]	2.33 (0.46-11.75)	0.307	9.66 (1.42-65.49)	0.020
Addition of bevacizumab	1.86 (0.86-4.01)	0.113	-	-
Sinusoidal dilatation				
absent	1		1	
Grade 1	0.40 (0.16-0.99)	0.049	0.32 (0.12-0.86)	0.024
Grade 2-3	0.07 (0.01-0.51)	0.009	0.05 (0.01-0.43)	0.006
Nodular regeneration				
Absent	1			
Grade 1	0.36 (0.05-2.80)	0.327	-	-
Grade 2-3*	N./A.	N./A.	-	-

[#] As reported as risk factor for steatosis in literature, irinotecan was forced into multivariable model; * the proportion of patients with grade 2-3 nodular regeneration is 100% for patients without severe steatosis and 0% for those with severe steatosis (Fisher's exact test: $p=0.200$); N./A., not applicable.

Table S4.2 Factors related to postoperative infectious complications.

Factors	Surgical site infections						Remote site infections						Systemic sepsis					
	univariable			multivariable			univariable			multivariable			univariable			multivariable		
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
<i>General characteristics</i>																		
Sex (male)	0.84 (0.47-1.51)	0.568	-	-	0.82 (0.41-1.62)	0.567	-	-	0.41 (0.16-1.03)	0.059	0.47 (0.17-1.29)	0.144	-	-	-	-	-	-
Age (year)	1.01 (0.98-1.04)	0.598	-	-	1.03 (0.99-1.07)	0.118	-	-	1.02 (0.97-1.07)	0.537	-	-	-	-	-	-	-	-
Body mass index (kg/m ²)	1.04 (0.97-1.12)	0.259	-	-	0.93 (0.85-1.03)	0.145	-	-	0.99 (0.87-1.12)	0.841	-	-	-	-	-	-	-	-
Comorbidity	1.08 (0.60-1.95)	0.805	-	-	2.26 (1.03-4.96)	0.042	2.59 (1.15-5.84)	0.022	1.77 (0.62-5.04)	0.283	-	-	-	-	-	-	-	-
Portal venous embolization	1.64 (0.48-5.65)	0.430	-	-	0.48 (0.06-3.86)	0.493	-	-	N/A.*	N/A.*	-	-	-	-	-	-	-	-
<i>Chemotherapy</i>																		
Preoperative chemotherapy	1.26 (0.67-2.36)	0.480	-	-	1.44 (0.67-3.10)	0.352	-	-	0.80 (0.31-2.10)	0.655	-	-	-	-	-	-	-	-
Addition of bevacizumab	0.99 (0.56-1.76)	0.976	-	-	1.48 (0.76-2.88)	0.251	-	-	1.69 (0.67-4.21)	0.265	-	-	-	-	-	-	-	-
<i>Surgical details</i>																		
Major hepatectomy (>3 segments)	1.61 (0.91-2.85)	0.099	1.47 (0.80-2.69)	0.215	1.92 (0.98-3.77)	0.059	1.95 (0.96-3.93)	0.064	0.81 (0.32-2.04)	0.647	-	-	-	-	-	-	-	-
Surgical duration (hour)	1.22 (1.04-1.43)	0.017	1.17 (0.99-1.37)	0.062	1.13 (0.96-1.33)	0.150	-	-	1.18 (0.98-1.43)	0.085	1.19 (0.96-1.49)	0.113	-	-	-	-	-	-
Pringle manoeuvre	1.52 (0.82-2.84)	0.185	-	-	1.66 (0.81-3.40)	0.163	-	-	1.29 (0.47-3.49)	0.622	-	-	-	-	-	-	-	-
Laparoscopic surgery	0.62 (0.20-1.88)	0.395	-	-	0.47 (0.11-2.08)	0.321	-	-	0.51 (0.07-3.97)	0.519	-	-	-	-	-	-	-	-
Perioperative blood transfusion	2.04 (1.04-4.01)	0.039	1.91 (0.93-3.95)	0.079	2.51 (1.18-5.31)	0.016	2.17 (0.99-4.77)	0.053	2.68 (1.01-7.13)	0.049	1.97 (0.64-6.11)	0.238	-	-	-	-	-	-

Table S4.2 (continued)

Factors	Surgical site infections						Remote site infections						Systemic sepsis					
	univariable			multivariable			univariable			multivariable			univariable			multivariable		
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
<i>Chemotherapy-associated liver injury</i>																		
Sinusoidal dilatation																		
Absent	1						1						1					
Grade 1	0.82 (0.42-1.59)	0.550	-	-	0.78 (0.33-1.86)	0.572	0.67 (0.28-1.64)	0.384	0.76 (0.27-2.15)	0.605	-	-	-	-	-	-	-	-
Grade 2-3	0.85 (0.42-1.73)	0.660	-	-	2.02 (0.93-4.37)	0.074	1.74 (0.77-3.89)	0.181	0.46 (0.12-1.71)	0.245	-	-	-	-	-	-	-	-
Steatosis																		
Absent	1						1						1					
Grade 1	1.68 (0.89-3.17)	0.109	1.71 (0.89-3.28)	0.109	1.15 (0.57-2.29)	0.702	-	-	4.07 (1.13-14.67)	0.032	4.38 (1.18-16.21)	0.027	-	-	-	-	-	-
Grade 2-3	2.74 (1.12-6.69)	0.027	3.55 (1.39-9.07)	0.008	0.39 (0.08-1.78)	0.222	-	-	5.44 (1.15-25.80)	0.033	6.73 (1.34-33.73)	0.020	-	-	-	-	-	-
Nodular regeneration																		
Absent	1						1						1					
Grade 1	0.62 (0.20-1.94)	0.412	-	-	0.97 (0.27-3.55)	0.967	-	-	0.66 (0.08-5.37)	0.700	-	-	-	-	-	-	-	-
Grade 2-3	0.47 (0.13-1.66)	0.239	-	-	1.95 (0.65-5.83)	0.234	-	-	0.70 (0.09-5.67)	0.736	-	-	-	-	-	-	-	-

N./A., not applicable; * the proportion of patients with portal venous embolization is 0% for patients with sepsis and 100% for those without sepsis (Fisher's exact test: p=0.764).

Table S4.3 Steatosis was related to superficial, but not severe surgical site infections.

Factors	Superficial surgical site infections			Severe surgical site infections		
	univariable	p-value	OR (95% CI)	multivariable	p-value	OR (95% CI)
General characteristics						
Sex (male)	1.09 (0.51-2.37)	0.821	-	-	0.67 (0.33-1.36)	0.265
Age (year)	1.01 (0.97-1.05)	0.592	-	-	1.01 (0.98-1.05)	0.480
Body mass index (kg/m ²)	1.09 (1.00-1.20)	0.055	1.05 (0.94-1.16)	-	1.01 (0.92-1.10)	0.913
Comorbidity	1.60 (0.71-3.60)	0.256	-	-	1.06 (0.51-2.19)	0.882
Portal venous embolization	0.63 (0.08-5.03)	0.661	-	-	2.15 (0.55-8.33)	0.269
Chemotherapy						
Preoperative chemotherapy	1.44 (0.62-3.33)	0.401	-	-	1.44 (0.64-3.20)	0.367
Addition of bevacizumab	1.12 (0.54-2.33)	0.763	-	-	1.01 (0.50-2.04)	0.976
Surgical details						
Major hepatectomy (≥ 3 segments)	0.89 (0.43-1.86)	0.759	-	-	2.59 (1.25-5.33)	0.010
Surgical duration (hour)	1.06 (0.88-1.28)	0.551	-	-	1.24 (1.04-1.48)	0.015
Pringle manoeuvre	1.33 (0.60-2.96)	0.488	-	-	1.74 (0.83-3.64)	0.145
Laparoscopic surgery	0.62 (0.14-2.75)	0.525	-	-	0.54 (0.12-2.38)	0.411
Perioperative blood transfusion	1.54 (0.65-3.65)	0.331	-	-	1.85 (0.83-4.14)	0.133
Chemotherapy-associated liver injury						
Sinusoidal dilatation	1					
Absent	1				1	
Grade 1	0.60 (0.25-1.44)	0.250	-	-	0.98 (0.43-2.23)	0.958
Grade 2-3	0.65 (0.26-1.66)	0.369	-	-	1.13 (0.48-2.65)	0.784
Steatosis						
Absent	1				1	
Grade 1	1.84 (0.75-4.48)	0.181	1.69 (0.68-4.21)	0.257	1.39 (0.66-2.95)	0.387
Grade 2-3	5.41 (1.87-15.65)	0.002	4.50 (1.43-14.10)	0.010	1.14 (0.34-3.78)	0.835
Nodular regeneration						
Absent	1				1	
Grade 1	0.34 (0.04-2.65)	0.302	-	-	0.75 (0.21-2.71)	0.664
Grade 2-3	1.20 (0.32-4.41)	0.789	-	-	N.A.*	N.A.*

N./A., not applicable; * the proportion of patients with severe surgical site infections is 100% for patients without grade 2-3 nodular regeneration and 0% for those with grade 2-3 nodular regeneration (Fisher's exact test p=0.050).

Table S4.4 Prognostic factors related to recurrence-free survival.

Factors	Recurrence-free survival					
	univariable		Multivariable (step-one)		Multivariable (step-two)	
	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
General characteristics						
Sex (male)	0.95 (0.67-1.36)	0.777	-	-	-	-
Age (year)	1.01 (0.99-1.02)	0.556	-	-	-	-
Body mass index (kg/m ²)	0.96 (0.91-1.00)	0.057	0.95 (0.91-0.99)	0.024	0.95 (0.91-0.99)	0.015
Comorbidity	1.17 (0.83-1.65)	0.383	-	-	-	-
Portal venous embolization	0.66 (0.30-1.41)	0.278	-	-	-	-
Primary colorectal tumour						
Rectal cancer	1.09 (0.77-1.52)	0.632	-	-	-	-
Tumour stage 3-4	1.34 (0.80-2.27)	0.270	-	-	-	-
Lymph node with tumour cells	1.20 (0.84-1.72)	0.319	-	-	-	-
Liver metastases						
Synchronous (<12 months)	1.44 (0.98-2.11)	0.063	1.43 (0.97-2.12)	0.070	1.43 (0.97-2.12)	0.133
Number of metastases	1.10 (1.02-1.20)	0.022	1.05 (0.96-1.15)	0.305	1.05 (0.95-1.15)	0.331
Diameter of largest metastases (cm)	1.04 (0.96-1.13)	0.350	-	-	-	-
Chemotherapy						
Preoperative chemotherapy	1.17 (0.80-1.70)	0.421	-	-	-	-
Addition of bevacizumab	0.97 (0.69-1.36)	0.863	-	-	-	-
Surgical details						
Major hepatectomy (≥3 Couinaud segments)	0.90 (0.64-1.26)	0.528	-	-	-	-
Surgical duration (hour)	1.04 (0.95-1.13)	0.395	-	-	-	-
Pringle manoeuvre	1.08 (0.72-1.61)	0.719	-	-	-	-
Laparoscopic surgery	1.01 (0.53-1.93)	0.971	-	-	-	-
Perioperative blood transfusion	1.03 (0.65-1.62)	0.911	-	-	-	-
Radical resection	0.49 (0.35-0.70)	<0.001	0.50 (0.35-0.73)	<0.001	0.50 (0.35-0.73)	<0.001
					0.54 (0.37-0.79)	0.001

Chapter 5

Impact of chemotherapy-associated liver injury on tumour regression grade and survival after partial hepatectomy for colorectal liver metastases

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Abstract

Introduction

An inverse relation between chemotherapy-associated liver injury (CALI) and tumour response to chemotherapy has been reported in literature. We aimed to validate these findings, and further investigate the impact of CALI on survival in patients who underwent partial hepatectomy for colorectal liver metastases (CRLM).

Methods

Patients who received neoadjuvant chemotherapy and underwent partial hepatectomy for CRLM between 2008 and 2014 in a tertiary referral centre were included. Liver and tumour specimens were histologically examined for CALI (steatosis, steatohepatitis, sinusoidal dilatation, nodular regeneration) and tumour regression grade (TRG). TRG 1-2 was defined as complete tumour response.

Results

166 consecutive patients were included with a median follow-up of 47 and 60 months for recurrence-free and overall survival, respectively. Grade 2-3 sinusoidal dilatation was found in 44 (26.5%) patients, grade 2-3 steatosis in 48 (28.9%), and steatohepatitis in one (0.6%) patient. With hardly observed liver fibrosis, nodular regeneration was present in 25 out of 133 patients (18.8%) with available collagen staining. Complete tumour response was observed in 33 (19.9%) patients. Grade 2-3 sinusoidal dilatation, but not steatosis or nodular regeneration, was associated with diminished complete tumour response (odds ratio 3.99, 95% CI 1.17-13.65, $p=0.027$). CALI was not significantly related to survival. Complete tumour response was associated with prolonged recurrence-free (hazard ratio 0.47, 95% CI 0.25-0.89, $p=0.020$) and overall survival (hazard ratio 0.35, 95% CI 0.18-0.68, $p=0.002$).

Conclusions

CALI was not directly related to survival. CALI was, however, associated with diminished complete tumour response, and tumour response, in turn, was associated with survival.

Introduction

Colorectal cancer is the third most common cancer worldwide, affecting over 1.3 million patients annually.¹ Over 50 percent of these patients develop colorectal liver metastases (CRLM).² Neoadjuvant chemotherapy is used to downstage the tumour and may facilitate future hepatic resection.^{3,4} Tumour downstaging relies on the tumour response to chemotherapy, as expressed in the tumour regression grade (TRG). CRLM patients with complete tumour response have been shown to have better survival than those with poor response.⁵

Systemic chemotherapy thus may have beneficial effects, but also frequently causes undesired liver parenchymal damage, collectively referred to as chemotherapy-associated liver injury (CALI). For instance, oxaliplatin-based treatment appears to be related to the development of sinusoidal obstruction syndrome⁶ and nodular regeneration,⁷ whereas irinotecan-based regimens are associated with increased occurrence of steatohepatitis.⁸⁻¹⁰ It has been shown by our group¹¹ and subsequently by others⁵ that CALI is associated with poor tumour response to neoadjuvant chemotherapy.

Despite the significant inverse relation between CALI and tumour response, few studies have been performed on this topic.^{5,11} Moreover, although one study claimed that chemotherapy-associated sinusoidal dilatation was associated with shortened survival,¹² another study could not reproduce this.⁵ Therefore, it remains unclear whether CALI influences survival. The first aim of the present study was to validate the relation between CALI and tumour response in an independent large cohort. The second aim was to explore whether sinusoidal dilatation, nodular regeneration, steatosis, and steatohepatitis were associated with survival in CRLM patients after partial hepatectomy.

Methods

Inclusion of patients

Consecutive patients who had undergone partial hepatectomy for CRLM at Maastricht University Medical Centre between January 2008 and December 2013 were considered for this study. Inclusion criteria were: 1) patients treated with neoadjuvant chemotherapy; 2) availability of adequate histopathology assessment of non-tumour

bearing liver tissue (i.e. presence of non-tumour-bearing liver at a distance of more than 2 cm from the tumour). Patients with cirrhosis were excluded from the study.

Definition and data collection

Comorbidity was defined as any disease affecting the patient apart from colorectal liver metastases (e.g. diabetes mellitus, and pulmonary, renal, cardiovascular, and other diseases). Overall morbidity was defined as any complication occurring within 30 days after surgery or during hospital stay and graded according to the classification of Dindo et al.¹³ Major morbidity was defined as Dindo-Clavien score IIIa (requiring invasive intervention) or higher. The concept of a liver surgery-specific complication was in correspondence to the liver surgery-specific composite endpoint (CEP) developed in 2011, and included one or more of the following events: ascites, postoperative liver failure, bile leakage, intra-abdominal abscess, intra-abdominal haemorrhage, and operative mortality.¹⁴ Data regarding postoperative infectious complications were prospectively collected daily by an independent infection-control nurse (MR) based on the following definitions. Postoperative infectious complications were defined as a combination of surgical site infections, remote site infections, and systemic sepsis. Surgical site infections were defined according to the guideline of the Centres for Disease Control and Prevention (CDC)'s recommendations, including superficial incisional, deep incisional, and intra-abdominal organ/space surgical site infections.¹⁵ Remote site infections were defined as a condition in which fever and leukocytosis were present with bacteria in urine, sputum, or central venous catheter tips according to an experienced physician's judgment with consideration of microbiologic evidence when available. Radical resection was defined as resected tumour lesions with a surgical margin of more than one millimeter, and confirmed to have absent tumour cells on the margin from routine pathology reports.

Pathology assessment of non-tumour-bearing liver and metastases

Sinusoidal dilatation, nodular regeneration, steatosis, and tumour regression grade were semi-quantitatively assessed by two experienced liver pathologists (AW and CV). Diagnosis of nodular regeneration was made only when confirming liver fibrosis was absent or minor. Assessment of steatohepatitis was performed by another experienced liver pathologist (JV) independently. All pathologists were blinded to clinical information concerning the patients.

The tissue was fixed in formalin, embedded in paraffin, and stained with hematoxylin & eosin and reticulin. Sinusoidal dilatation was graded according to Rubbia-Brandt et al.¹⁶

Nodular regeneration was graded according to the Wanless scoring system.¹⁷ Steatosis was graded according to Kleiner et al.¹⁸ A case presenting with at least grade 1 of each of the three features (steatosis, hepatocellular ballooning, and lobular inflammation) was classified as steatohepatitis.¹⁹ Tumour regression was graded as described by Mandard et al,²⁰ for the assessment of tumour regression after preoperative chemoradiotherapy for esophageal carcinoma, and modified for liver metastases. Grade 1 is characterized by the absence of histologically identifiable residual tumour and extensive fibrosis; grade 2 shows the presence of rare residual tumour cells scattered through the fibrosis; grade 3 represents a substantial amount of residual tumour cells but fibrosis dominated; grade 4 reflects residual tumour cells outgrowing fibrosis; and grade 5 indicates the absence of any tumour regression.

Statistical Analyses

All statistical analyses were performed using IBM SPSS Statistics for Windows (version 20.0). The relationship between CALI and tumour regression grade was studied applying uni- and multivariable binary logistic regression models. Furthermore, the association between CALI, tumour regression grade, infectious complications, and survival was analyzed using uni- and multivariable Cox regression models. Variables with p -value ≤ 0.10 in univariable analysis were included in the multivariable analysis. Odds ratios (OR) or hazard ratios (HR) with 95% confidence intervals (CI) were calculated. A p -value ≤ 0.05 was considered significant for all tests.

Results

A total of 166 consecutive patients were included (Table 5.1). Of these patients, 103 (62.0%) were male and the median age was 65 (interquartile range 32-85) years. Preoperative comorbidity was found in 106 (63.9%) patients. Nine (5.4%) patients had undergone portal venous embolization before liver surgery. Ninety-nine (59.6%) patients had more than one liver metastasis. Capecitabine in combination with oxaliplatin (CAPOX) was the most commonly used regimen, which was administered to 152 (91.6%) patients. Bevacizumab was administered to 105 (63.3%) patients. Postoperative surgical site infections were present in 41 (30.7%) patients, and remote site infections were found in 26 (15.7%) patients.

Table 5.1 Clinical characteristics.

Factors	n=166
General characteristics	
Sex (male)	103 (62.0%)
Age (year)	65 (58-69)
Body mass index (kg/m ²)	25.9 (23.8-28.3)
Comorbidity	106 (63.9%)
Diabetes	13 (7.8%)
Cardiovascular	70 (42.2%)
Pulmonary	18 (10.8%)
Renal	6 (3.6%)
Rest	39 (23.5%)
Portal venous embolization	9 (5.4%)
Primary colorectal tumour	
Rectal cancer	72 (43.4%)
Tumour stage 3-4 (n=165*)	144 (87.3%)
Lymph node with tumour cells (n=162*)	113 (69.8%)
Liver metastases	
Synchronous metastases (<12 months)	130 (78.3%)
Number of metastases	2 (1-3)
Diameter of largest metastases	2.5 (1.3-4.0)
CEA >200 ng/ml at hepatectomy	5 (3.0%)
Chemotherapy details	
5-fluorouracil	6 (3.6%)
Oxaliplatin	153 (92.2%)
Capecitabine	152 (91.6%)
Irinotecan	8 (4.8%)
Bevacizumab	105 (63.3%)
Surgical details	
Major hepatectomy (≥3 Couinaud segments)	78 (47.0%)
Pringle manoeuvre	44 (26.5%)
Laparoscopic surgery	11 (6.6%)
Perioperative blood transfusion	32 (19.3%)
Radical resection	111 (66.9%)
Postoperative characteristics	
Overall morbidity	76 (45.8%)
Major morbidity (Dindo-Clavien III-V)	29 (17.5%)
Liver surgery-specific complications	32 (19.3%)
Infectious complications	51 (30.7%)
Surgical site infections	41 (24.7%)
Superficial wound	22 (13.3%)
Deep wound	6 (3.6%)
Intraabdominal organ/space	27 (16.3%)
Remote site infections	26 (15.7%)
Urinary tract	12 (7.2%)
Respiratory tract	15 (9.0%)
Central venous catheter-related	6 (3.6%)
Sepsis	13 (7.8%)
Ninety-day mortality	5 (3.0%)
Length of hospital stay (day)	8 (6-10)

Numeric variables are presented as median with interquartile range; *Due to missing data, indicated number of patients was below 166; CEA, carcinoembryonic antigen.

Table 5.2 summarizes the pathology details of chemotherapy-associated liver injury. Of the 90 (54.2%) patients who presented with sinusoidal dilatation, mild lesions (grade 1) were found in 46 (27.7%), and severe lesions (grade 2 or grade 3) in 44 (26.5%) patients. Mild steatosis (grade 1) was found in 56 (33.7%), and severe steatosis (grade 2 and grade 3) in 48 (28.9%) patients. Collagen staining was available from 133 patients, of which nodular regeneration was found in 24 (18.5%) patients. Steatohepatitis was present in one (0.6%) patient, who had grade 2 steatosis, grade 2 hepatocellular ballooning, and grade 1 lobular inflammation. Complete tumour response (i.e. tumour regression grade 1-2) was observed in 33 (19.8%) patients.

Table 5.2 Pathology characteristics.

Pathology details	n=166
Sinusoidal dilatation	
Absent	76 (45.8%)
Grade 1	46 (27.7%)
Grade 2	30 (18.1%)
Grade 3	14 (8.4%)
Nodular regeneration (n=133*)	
Absent	108 (65.1%)
Grade 1	11 (6.6%)
Grade 2	12 (7.2%)
Grade 3	2 (1.5%)
Steatosis	
Absent	62 (37.3%)
Grade 1	56 (33.7%)
Grade 2	29 (17.5%)
Grade 3	19 (11.4%)
Steatohepatitis	1 (0.6%)
Tumour regression grade	
Grade 1	20 (12.0%)
Grade 2	13 (7.8%)
Grade 3	47 (28.3%)
Grade 4	56 (33.7%)
Grade 5	30 (18.1%)

*Due to unavailable collagen staining, indicated number of patients was below 166.

The impact of CALI and other potentially important factors on tumour regression grade is depicted in Figure 5.1 (Detailed data are summarized in Supplemental Table S5.1). Given the fact that only one case of steatohepatitis was found, the relation between steatohepatitis and tumour regression grade could not be studied. Severe sinusoidal dilatation was associated with increased occurrence of diminished tumour response (OR 3.99, 95% CI 1.17-13.65, $p=0.027$). An association between mild sinusoidal dilatation and diminished tumour response was statistically not significant (OR 2.38, 95% CI 0.83-6.85, $p=0.108$). Addition of bevacizumab to the chemotherapy treatment tended to be

associated with increased complete tumour response (OR 0.35, 95% CI 0.12-1.01, $p=0.052$). Severe steatosis (OR 0.85, 95% CI 0.33-2.21, $p=0.741$) and severe nodular regeneration (OR 1.88, 95% CI 0.39-9.12, $p=0.434$) were not related to tumour regression grade. In subgroup analyses, an inverse relation between the addition of bevacizumab and occurrence of severe sinusoidal dilatation (OR 0.35, 95% CI 0.17-0.76, $p=0.008$) was observed. Similarly, presence of severe steatosis was also inversely related to that of severe sinusoidal dilatation (OR 0.23, 95% CI 0.08-0.66, $p=0.006$) (Supplemental Table S5.2).

Figure 5.1 Factors related to tumour response.

Data are depicted as odds ratios (symbols) with 95% confidence intervals (bars) based on results of multivariable analysis; asterisks indicate p -value ≤ 0.05 .

The relation between potential prognostic factors and recurrence-free survival is summarized in Figure 5.2 (Detailed data are summarized in Supplemental Table S5.3). Median follow-up for recurrence-free survival was 47 months. Both complete tumour response (HR 0.47, 95% CI 0.25-0.89, $p=0.020$) and radical resection (HR 0.58, 95% CI 0.38-0.88, $p=0.011$) were associated with prolonged recurrence-free survival. Sinusoidal dilatation, steatosis, and nodular regeneration were not significantly associated with recurrence-free survival. The presence of remote site infections was the sole factor independently related to shortened recurrence-free survival (HR 1.87, 95% CI 1.06-3.30, $p=0.030$).

Figure 5.2 Prognostic factors related to recurrence-free survival. Data are depicted as hazard ratios (symbols) with 95% confidence intervals (bars) based on results of multivariable analysis, asterisks indicate p -value ≤ 0.05 .

Figure 5.3 summarizes the association between potential prognostic factors and overall survival (Detailed data are summarized in Supplemental Table S5.4). Median follow-up for overall survival was 60 months. Complete tumour response (i.e. tumour regression grade 1-2, HR 0.35, 95% CI 0.18-0.68, $p=0.002$) and radical resection (HR 0.52, 95% CI 0.34-0.80, $p=0.003$) were associated with prolonged overall survival. Again, sinusoidal dilatation, steatosis, and nodular regeneration were not significantly associated with overall survival. Synchronous (<12 months) liver metastases (HR 1.98, 95% CI 1.14-3.47, $p=0.016$) and remote site infections (HR 1.87, 95% CI 1.06-3.30, $p=0.030$) were independently related to shortened overall survival.

Figure 5.3 Prognostic factors related to overall survival. Data are depicted as hazard ratios (symbols) with 95% confidence intervals (bars) based on results of multivariable analysis; asterisks indicate p-value ≤ 0.05 .

Discussion

The data of the present study demonstrate that poor tumour response after neoadjuvant chemotherapy occurred more often in patients with sinusoidal dilatation, whereas neither steatosis nor nodular regeneration was associated with tumour response. Complete tumour response, in turn, was a strong independent predictor of prolonged recurrence-free survival and overall survival. However, none of the studied background liver abnormalities was independently related to survival. Addition of bevacizumab to chemotherapy was associated with decreased occurrence of sinusoidal dilatation, and tended to be related to increased complete tumour response. Moreover, remote site infections were related to reduced recurrence-free survival and overall survival, whereas radical resection was associated with an increase for these outcome parameters.

Although sinusoidal dilatation was associated with diminished complete tumour response, which was associated with recurrence-free survival and overall survival, sinusoidal dilatation itself was not directly related to survival. As neoadjuvant chemotherapy provides increased complete tumour response,²¹ and this is related to prolonged survival, this observation might indicate that the benefits of neoadjuvant chemotherapy in terms of prolonging survival outweigh the harm of chemotherapy-induced undesired parenchymal liver injury. It is also suggested that chemotherapy could be more beneficial if clinicians could reduce the negative impact of CALI on tumour response. Since addition of bevacizumab to the chemotherapy treatment was associated with both a lower prevalence of sinusoidal dilatation and a tendency to increased tumour response, it seems beneficial to add bevacizumab to oxaliplatin-based chemotherapy.

In the present study, the previously shown^{5,11} inverse relation between chemotherapy-associated sinusoidal dilatation and tumour regression grade was confirmed. There may be several explanations for this finding. Loss of fenestrae in sinusoidal endothelial cells, which has been shown in an animal model of sinusoidal obstruction syndrome,²² might inhibit the transport of compounds²³ such as oxaliplatin to the tumour, thereby limiting chemotherapy exposure. Oxaliplatin based chemotherapy is also known to lead to the detachment of sinusoidal endothelial cells from the space of Disse²⁴, which may further weaken the hepatic microcirculation and limit transportation of drugs to the tumour.^{24,25} Moreover, a compromised hepatic microcirculation will lead to hypoperfusion and may induce a state of hepatic hypoxia.²⁶ Tumour hypoxia will subsequently stimulate angiogenesis that may promote tumour cell invasion.²⁷ Taken together, loss of fenestrae from sinusoidal endothelial cells, a weakened hepatic microcirculation, and hepatic hypoxia related to sinusoidal dilatation are all potential contributors to a diminished tumour response.

In line with a previous report²⁸, the present study shows a negative relation between the presence of remote site infections and recurrence-free survival as well as overall survival. Although the mechanisms underlying this observation remain unclear, and a specific cause-effect relationship cannot be proven, we hypothesize that innate or adaptive immunity may play an important role. Patients with immune suppression suffer from increased occurrence of surgical infections.²⁹ In keeping with this, enhancing the immune system by addition of thymosin³⁰ inhibits tumour progression,³¹ and may enhance tumour response and prolong survival.³² In essence, the presence of infections may reflect a relatively weaker immune system. Weakened immunity may reduce

tumour suppression and may eventually result in more frequent or earlier tumour recurrence and shortened survival.

Some limitations of this study should be discussed. Although analysis of the effect of steatohepatitis on tumour regression grade and survival would have been of interest, this was not possible since only one patient was diagnosed with steatohepatitis. This very low incidence of steatohepatitis could be due to the fact that only a small number of patients were treated with irinotecan, a known risk factor for the development of steatohepatitis.⁸⁻¹⁰ Next, data on the interval between cessation of neoadjuvant chemotherapy and partial hepatectomy, as well as the number of administered cycles, were not available for the majority of included patients, since chemotherapy was frequently administered at local referral hospitals. Therefore, the influence of these factors on tumour regression grade and survival could not be evaluated. Despite this limitation, it must be highlighted that the majority of the studied population received the same regimen, which was capecitabine in combination with oxaliplatin. Therefore, the effect of treatment regimen related factors on tumour regression grade and survival was presumably minimal.

Conclusion

This study demonstrated that chemotherapy-associated sinusoidal dilatation is related to diminished complete tumour response after neoadjuvant chemotherapy. Complete tumour response was shown to be the strongest predictor of prolonged recurrence-free survival and overall survival. None of the background parenchymal chemotherapy-induced liver injuries was associated with survival. Addition of bevacizumab was associated with decreased sinusoidal dilatation, and tended to be related to increased complete tumour response. Given that oxaliplatin-based treatment was associated with increased sinusoidal dilatation,⁶ and that sinusoidal dilatation was related to diminished tumour response, it seems advisable to co-administer bevacizumab with oxaliplatin-based regimens. Furthermore, since infections were related to reduced survival, application of meticulous aseptic surgery techniques and infection prevention protocols may be of critical importance for improving long-term outcome.

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Supplemental tables

Table S5.1 Factors related to tumour response.

Factors	Tumour regression grade 3-5			
	univariable		multivariable	
	OR (95% CI)	p-value	OR (95% CI)	p-value
General characteristics				
Sex (male)	1.43 (0.65-3.14)	0.371	-	-
Age (year)	1.00 (0.96-1.04)	0.983	-	-
Body mass index (kg/m ²)	1.00 (0.90-1.12)	0.941	-	-
Comorbidity	0.47 (0.19-1.12)	0.087	0.32 (0.12-0.86)	0.024
Portal venous embolization	n.a.*	n.a.*	-	-
Primary colorectal tumour				
Rectal cancer	0.60 (0.27-1.31)	0.197	-	-
Tumour stage 3-4	2.16 (0.78-6.02)	0.139	-	-
Lymph node with tumour cells	0.64 (0.26-1.56)	0.323	-	-
Liver metastases				
Synchronous (<12 months)	0.25 (0.07-0.88)	0.032	0.33 (0.09-1.30)	0.114
Number of metastases	1.02 (0.84-1.24)	0.850	-	-
Diameter of largest metastases (cm)	1.29 (1.02-1.64)	0.037	1.13 (0.89-1.43)	0.326
CEA > 200 ng/ml at hepatectomy	n.a.†	n.a.†	-	-
Chemotherapy				
Addition of bevacizumab	0.30 (0.12-0.80)	0.015	0.35 (0.12-1.01)	0.052
Chemotherapy-associated liver injury				
Sinusoidal dilatation				
Absent	1		1	
Grade 1	2.46 (0.92-6.58)	0.073	2.38 (0.83-6.85)	0.108
Grade 2-3	4.36 (1.44-13.21)	0.009	3.99 (1.17-13.65)	0.027
Steatosis				
Absent	1			
Grade 1	0.97 (0.37-2.50)	0.942	-	-
Grade 2-3	0.85 (0.33-2.21)	0.741	-	-
Nodular regeneration	2.30 (0.63-8.43)	0.208	-	-
Absent	1			
Grade 1	3.14 (0.38-26.17)	0.289	-	-
Grade 2-3	1.88 (0.39-9.12)	0.434	-	-

*The proportion of patients with complete tumour response is 0% for patients with portal venous embolization and 100% for those without (Fisher's exact test: $p=0.258$; †the proportion of patients with complete tumour response is 0% for patients with CEA >200ng/ml and 100% for those with CEA ≤200ng/ml (Fisher's exact test: $p=0.650$); CEA, carcinoembryonic antigen.

Table S5.2 Factors related to sinusoidal dilatation.

Factors	Sinusoidal dilatation grade 2-3			
	univariable		multivariable	
	OR (95% CI)	p-value	OR (95% CI)	p-value
General characteristics				
Sex (male)	1.16 (0.56-2.41)	0.693	-	-
Age (year)*	1.03 (0.99-1.08)	0.101	1.04 (1.00-1.08)	0.070
Body mass index (kg/m2)	1.01 (0.92-1.12)	0.824	-	-
Comorbidity	1.46 (0.68-3.12)	0.331	-	-
Portal venous embolization	2.20 (0.49-9.96)	0.307	-	-
Chemotherapy				
Oxaliplatin	1.33 (0.27-6.61)	0.732	-	-
Capecitabine	0.84 (0.24-2.95)	0.790	-	-
Irinotecan	2.49 (0.57-10.82)	0.223	-	-
5-fluorouracil	0.90 (0.16-5.21)	0.906	-	-
Addition of bevacizumab	0.34 (0.16-0.71)	0.004	0.35 (0.17-0.76)	0.008
Chemotherapy-associated liver injury				
Steatosis				
Absent	1		1	
Grade 1	0.55 (0.24-1.26)	0.156	0.50 (0.19-1.09)	0.077
Grade 2-3	0.24 (0.09-0.65)	0.005	0.23 (0.08-0.66)	0.006
Nodular regeneration (n=133)‡¶				
Absent	1		-	
Grade 1	3.32 (0.87-12.62)	0.079	-	-
Grade 2-3	11.05 (2.90-42.11)	0.0004	-	-

*Considering the p-value in univariable analysis reached borderline of 0.10, age was included in the multivariable analysis; ‡Due to missing data, indicated number of patients was below 166; ¶Because of the suspected collinearity between sinusoidal dilatation and nodular regeneration, nodular regeneration was not included into multivariable analysis.

Table S5.3 Prognostic factors related to recurrence-free survival.

Factors	Recurrence-free survival			
	univariable		multivariable	
	HR (95% CI)	p-value	HR (95% CI)	p-value
General characteristics				
Sex (male)	1.07 (0.70-1.63)	0.768	-	-
Age (year)	1.00 (0.98-1.02)	0.958	-	-
Body mass index (kg/m ²)	0.95 (0.89-1.01)	0.079	0.94 (0.89-1.00)	0.067
Comorbidity	1.30 (0.86-1.98)	0.219	-	-
Portal venous embolization	0.86 (0.35-2.12)	0.734	-	-
Primary colorectal tumour				
Rectal cancer	1.15 (0.77-1.72)	0.496	-	-
Tumour stage 3-4	1.65 (0.88-3.11)	0.122	-	-
Lymph node with tumour cells	1.37 (0.88-2.14)	0.169	-	-
Liver metastases				
Synchronous (<12 months)	1.42 (0.86-2.33)	0.168	-	-
Number of metastases	1.06 (0.95-1.18)	0.297	-	-
Diameter of largest metastases (cm)	1.02 (0.93-1.12)	0.695	-	-
CEA >200 ng/ml at hepatectomy	1.92 (0.78-4.74)	0.158	-	-
Chemotherapy				
Addition of bevacizumab	0.87 (0.57-1.32)	0.508	-	-
Surgical details				
Major hepatectomy (≥3 Couinaud segments)	0.79 (0.53-1.18)	0.245	-	-
Pringle manoeuvre	1.08 (0.69-1.70)	0.737	-	-
Laparoscopic surgery	1.30 (0.56-2.99)	0.544	-	-
Perioperative blood transfusion	1.14 (0.67-1.94)	0.639	-	-
Radical resection	0.52 (0.34-0.78)	0.002	0.58 (0.38-0.88)	0.011
Postoperative morbidity				
Overall morbidity	1.40 (0.93-2.10)	0.105	-	-
Major morbidity (Dindo-Clavien III-V)	1.55 (0.88-2.70)	0.127	-	-
Liver surgery-specific complications	1.72 (1.04-2.83)	0.034	1.42 (0.82-2.44)	0.207
Surgical site infections	1.30 (0.82-2.07)	0.266	-	-
Remote site infections	2.29 (1.38-3.81)	0.001	2.12 (1.17-3.86)	0.014
Chemotherapy-associated liver injury				
Sinusoidal dilatation				
Absent	1		1	
Grade 1	1.59 (0.98-2.58)	0.060	1.21 (0.73-2.00)	0.454
Grade 2-3	1.41 (0.83-2.42)	0.208	0.87 (0.47-1.62)	0.668
Steatosis				
Absent	1			
Grade 1	1.15 (0.71-1.86)	0.566	-	-
Grade 2-3	0.96 (0.59-1.59)	0.886	-	-
Nodular regeneration				
Absent	1			
Grade 1	0.68 (0.29-1.58)	0.366	-	-
Grade 2-3	1.02 (0.51-2.05)	0.946	-	-
Tumour response				
Tumour regression grade				
Grade 4-5	1			
Grade 3	0.97 (0.61-1.55)	0.907	0.79 (0.49-1.28)	0.337
Grade 1-2	0.51 (0.28-0.92)	0.026	0.47 (0.25-0.89)	0.020

CEA, carcinoembryonic antigen.

Table S5.4 Prognostic factors related to overall survival.

Factors	Overall survival			
	univariable		multivariable	
	HR (95% CI)	p-value	HR (95% CI)	p-value
General characteristics				
Sex (male)	1.04 (0.69-1.55)	0.867	-	-
Age (year)	1.02 (1.00-1.04)	0.064	1.02 (0.99-1.04)	0.180
Body mass index (kg/m ²)	0.96 (0.91-1.02)	0.165	-	-
Comorbidity	1.10 (0.73-1.64)	0.655	-	-
Portal venous embolization	0.98 (0.40-2.43)	0.972	-	-
Primary colorectal tumour				
Rectal cancer	0.98 (0.66-1.45)	0.919	-	-
Tumour stage 3-4	1.52 (0.79-2.92)	0.214	-	-
Lymph node with tumour cells	1.21 (0.78-1.87)	0.390	-	-
Liver metastases				
Synchronous (<12 months)	1.67 (1.00-2.79)	0.051	1.98 (1.14-3.47)	0.016
Number of metastases	1.10 (1.00-1.21)	0.050	1.06 (0.96-1.18)	0.245
Diameter of largest metastases (cm)	1.01 (0.92-1.11)	0.906	-	-
CEA >200 ng/ml at hepatectomy	2.67 (1.08-6.58)	0.064	2.59 (0.97-6.92)	0.058
Chemotherapy				
Addition of bevacizumab	0.94 (0.63-1.40)	0.747	-	-
Surgical details				
Major hepatectomy (≥3 Couinaud segments)	0.80 (0.54-1.19)	0.268	-	-
Pringle manoeuver	1.03 (0.66-1.61)	0.885	-	-
Laparoscopic surgery	0.76 (0.31-1.86)	0.544	-	-
Perioperative blood transfusion	1.06 (0.65-1.73)	0.815	-	-
Radical resection	0.44 (0.30-0.66)	<0.001	0.52 (0.34-0.80)	0.003
Postoperative morbidity				
Overall morbidity	1.33 (0.90-1.96)	0.156	-	-
Major morbidity (Dindo-Clavien III-V)	1.16 (0.70-1.93)	0.568	-	-
Liver surgery-specific complications	1.49 (0.94-2.38)	0.092	1.14 (0.69-1.91)	0.605
Surgical site infections	1.34 (0.86-2.10)	0.195	-	-
Remote site infections	1.79 (1.08-2.97)	0.023	1.87 (1.06-3.30)	0.030
Chemotherapy-associated liver injury				
Sinusoidal dilatation				
Absent	1			
Grade 1	1.54 (0.95-2.47)	0.078	1.18 (0.72-1.95)	0.509
Grade 2-3	1.59 (0.97-2.60)	0.067	1.08 (0.63-1.85)	0.789
Steatosis				
Absent	1			
Grade 1	1.20 (0.75-1.93)	0.447	-	-
Grade 2-3	0.86 (0.52-1.42)	0.555	-	-
Nodular regeneration			-	-
Absent	1			
Grade 1	1.19 (0.56-2.51)	0.651	-	-
Grade 2-3	0.79 (0.38-1.64)	0.518	-	-
Tumour response				
Tumour regression grade				
Grade 4-5	1		1	
Grade 3	0.73 (0.46-1.14)	0.167	0.69 (0.43-1.11)	0.129
Grade 1-2	0.34 (0.18-0.64)	0.001	0.35 (0.18-0.68)	0.002

CEA, carcinoembryonic antigen.

Part II

Mechanisms underlying chemotherapy
-associated liver injury

Chapter 6

miR-21 and miR-150 downregulation is associated with
oxaliplatin-induced sinusoidal dilatation and
impaired survival

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Abstract

Background

Oxaliplatin-induced sinusoidal dilatation (SD) is associated with increased morbidity and impaired survival. Hence, identification of the molecular mechanisms underpinning the development of SD is pivotal.

Methods

This case-matched cohort study of patients who underwent partial hepatectomy for colorectal liver metastases between 2008 and 2009 included 28 patients with preoperative oxaliplatin treatment and SD (grade 2-3 *versus* absent); ten patients with grade 2-3 SD were matched with ten without SD by number of oxaliplatin cycles, cumulative oxaliplatin dose, sex, age, body mass index, and liver transaminases. Non-tumour liver microRNA expression was analyzed using Agilent arrays for the 20 patients that could be matched and validated by quantitative PCR for all 28 patients included. MicroRNA expression was subsequently related to overall survival and recurrence free survival.

Results

Grade 2-3 SD was not associated with upregulation of any microRNA. However, miR-21 and miR-150 were significantly downregulated in the grade 2-3 SD group (array: 1.34-fold, $p=0.028$; 1.46-fold, $p=0.035$, respectively; qPCR: 1.32-fold, $p=0.001$; 1.34-fold, $p=0.014$, respectively). Low hepatic expression of both miR-21 and miR-150 was associated with impaired overall survival ($n=28$, $p=0.032$ and $p=0.010$, respectively). Likewise, both overall recurrence-free survival and liver recurrence-free survival were reduced in subjects with low hepatic miR-21 ($p=0.010$ and $p=0.046$, respectively) and miR-150 expression ($p=0.025$ and $p=0.031$, respectively).

Conclusions

The strong association between reduced hepatic miR-21/miR-150 levels and SD, tumour recurrence, and survival indicates that miR-21 and miR-150 regulated genes may be involved in the pathogenesis of SD and may be used as prognostic markers.

Introduction

Colorectal cancer is the third most common cancer in men and second in women worldwide, affecting over 1.3 million patients annually.¹ The liver is the dominant metastatic site, and approximately 50% of patients develop colorectal liver metastases (CRLM).² Although liver resection is the only potentially curative treatment, only 10-30% of patients with liver metastases are candidates for hepatic surgery.² Inoperability is mainly caused by patient comorbidity in combination with number and size of hepatic tumour. For patients who have unresectable metastases, chemotherapy is currently the most commonly used treatment to prolong survival.

For decades, 5-fluorouracil was the sole active chemotherapeutic agent for CRLM. This has changed markedly since 2000: with the approval of irinotecan, oxaliplatin, and humanized monoclonal antibodies, many more initially unresectable CRLM became resectable.³ Although response rates are convincing administration of these agents is associated with significant liver injury. More specifically, the administration of irinotecan appears to be related to the development of nonalcoholic steatohepatitis, whilst oxaliplatin may lead to the development of sinusoidal obstruction syndrome.⁴

Chemotherapy-associated sinusoidal obstruction syndrome in patients with CRLM is characterized by sinusoidal dilatation (SD), centrilobular vein injury, centrilobular fibrosis, and peliosis.⁵ SD is the hallmark feature and routinely used to evaluate the severity of sinusoidal obstruction syndrome.⁵ We and others previously showed that the development of SD diminishes the tumour response to chemotherapy.^{6,7} SD has also been shown to increase postoperative morbidity⁸ and compromise postoperative recurrence-free survival and overall survival in patients undergoing liver surgery for CRLM.⁹

In view of these clinical observations and the lack of treatment options for SD, it is of key importance to identify the molecular mechanisms underlying its development. Recent mRNA microarray-based approaches showed potential involvement of angiogenesis, coagulation, the acute phase response, hepatic fibrosis, oxidative stress, and hypoxia in SD.¹⁰ However, expression profiles of microRNAs, which function as regulators of gene expression through translational repression or mRNA cleavage, have not yet been investigated in the context of SD. Nevertheless, microRNAs are known to play important roles in tumour angiogenesis and progression¹¹ and have been linked to survival in patients with colorectal cancer.¹² Furthermore, the concentration of several microRNAs has been shown to be enriched in the serum of patients with drug-induced liver injury.¹³

Moreover, specific circulating microRNAs have been shown to represent reliable biomarkers for predicting prognosis and the tumour response to chemotherapeutic drugs.^{14,15} In addition, targeting a specific microRNA involved in the pathogenesis of liver disease is a feasible and effective treatment option.¹⁶ Therefore, we aimed to obtain novel insight into the pathogenesis of SD by identifying hepatic microRNAs associated with the development of SD, and by studying the links between these microRNAs and tumour recurrence as well as survival.

Materials and methods

Patients and sample selection

A total of 40 patients who underwent partial hepatectomy for CRLM and had preoperative oxaliplatin-based chemotherapy at Maastricht University Medical Centre between Jan 2008 and Dec 2009 were included in the study. Plasma was collected after general anesthesia was induced, but before liver surgery. Non-tumour liver biopsies (>2 cm distance from the tumour) were taken before manipulation of the liver. The study was approved by the Medical Ethics Committee of Maastricht University Medical Centre and conducted according to the revised version of the Declaration of Helsinki. Written informed consent was obtained from each subject prior to study participation.

Assessment of sinusoidal injury

Formalin-fixed, paraffin-embedded archived samples of the non-tumour liver parenchyma were reviewed by two experienced liver pathologists (CV and AD). In case of dissent, final consensus was made after discussion. Morphology analysis was based on hematoxylin and eosin (H&E), reticulin, and Masson Trichrome stained slides. Histopathology features were scored according to the classification of Rubbia-Brandt and colleagues.⁵ Based on these criteria, SD was semi-quantified from 0 to 3: 0, absent (n=14); 1, mild (centrilobular involvement limited to one third of the lobular surface, n=12); 2, moderate (centrilobular involvement extended to two thirds of the lobular surface, n=11); and 3, severe (the whole lobule involved, n=3).

Microarray analyses

A well-matched population of patients with grade 2-3 SD and patients without SD (grade 0) was selected for investigating microRNA expression. The matching parameters were: number of oxaliplatin cycles, cumulative oxaliplatin dose, sex, age, body mass index, and preoperative alanine transaminase and aspartate transaminase concentrations. Snap-

frozen liver biopsy specimens from the 20 patients who could be matched were cut into small pieces, of which the largest dimension was less than 0.5 cm. Samples were immediately soaked in pre-chilled *RNAlater* (Life Technologies, Bleiswijk, NL) and kept for at most 24 hours at 4°C. *RNAlater*-stabilized liver tissue was homogenized in 700 µl QIAzol Lysis Reagent (Qiagen, Venlo, NL) according to the manufacturer's protocol. RNA purification procedures were carried out with the miRNeasy Mini Kit (Qiagen, Venlo, NL) as instructed by the manufacturer. The quality of purified RNA was assessed using the Bioanalyser 2100 (Agilent Technologies, Amstelveen, NL). Samples with an RNA integrity number (RIN) > 6.0 were used for cDNA preparation and hybridization.

With an input of 100ng RNA, cDNA targets were prepared by following the protocol for the microRNA microarray system with the microRNA complete labeling and hyb kit (Agilent, version 2.4, September 2011). MicroRNA expression profiling was performed using Agilent Sureprint G3 Human V19 microRNA 8 x 60K microarrays. The hybridization was performed according to the manufacturer's protocol; afterwards, the microarray slides were washed and scanned with a DNA microarray scanner (Agilent Technologies, Amstelveen, NL). The scanned images were converted into TXT files using the Feature Extraction Software v10.7.3.1 from Agilent Technologies, and were imported in an in-house developed pipeline for quality control using R 2.15.2 (<http://www.r-project.org>). Further downstream analysis was performed by means of MagiCMicroRna.¹⁷ Total microRNA signals were log2 transformed and quantile normalized. The MagiCMicroRna filtering procedure was based on subsets of experimental conditions (grade 2-3 *versus* grade 0 SD). Gene Expression Omnibus (GEO) number to be assigned.

Quantitative PCR analysis of liver specimens

To validate the results from the microarray, we performed a quantitative PCR analysis of the 20 samples analyzed by microarray as well as 8 additional samples of patients with SD grade 0, 2, or 3 that could not be matched for the above-mentioned parameters. Snap-frozen liver specimens were homogenized in Lysis/Binding Buffer provided by the mirVana miRNA Isolation Kit (Life technologies, Bleiswijk, NL). Total RNA was isolated according to the manufacturer's protocol. cDNA was synthesized from 1.7µg of total RNA using the miScript II RT Kit (Qiagen, Venlo, NL). The procedures were performed according to the manufacturer's protocol. Afterwards, quantitative PCR was performed with the SensiMix SYBR & Fluorescein Mastermix (Bioline, Alphen aan den Rijn, NL) and the appropriate miScript Primer Assay (Qiagen) in an iQ5 iCycler (Bio-Rad, Veenendaal, NL) according to the manufacturer's instructions. The quantitative PCR protocol was: 95°C for 15 minutes, followed by 40 cycles of 95°C for 10 seconds, 60°C for 20 seconds, and 70°C for 20 seconds. Relative miR-21 and miR-150 expression was calculated with

LinregPCR software (version 2014.2, Heart Failure Research Centre, Amsterdam, NL) using miR-16 as a stably-expressed reference microRNA. Sequences of the applied PCR primers are listed in Supplemental Table S6.1 (supporting information).

Quantitative PCR analysis of plasma specimens

To explore the potential association between hepatic microRNA expression and microRNA concentration in systemic blood (plasma), we also performed quantitative PCR of EDTA treated plasma from the patients included for quantitative PCR analysis (one patient with unavailable plasma was excluded). Total RNA was isolated from 200µl plasma using the miRNeasy Serum/Plasma Kit (Qiagen, Venlo, NL), and subsequently eluted in 14µl RNase-free water according to the manufacturer's instructions. 9µl extracted RNA was used (per 20µl reaction) to synthesize cDNA using the miScript II RT Kit (Qiagen, Venlo, NL). The 20µl-reaction mix was further diluted 11 times in nuclease-free water, and 5µl of cDNA was used per quantitative PCR using SensiMix SYBR & Fluorescein Mastermix (Bioline, Alphen aan den Rijn, NL) and an iQ5 iCycler (Bio-Rad, Veenendaal, NL). Data were normalized for isolation efficiency using a mix of 3 spiked-in synthetic *Caenorhabditis elegans* microRNAs,¹⁸ lacking sequence homology to human microRNAs. Oligonucleotides (2µl; 4.8×10^8 copies/µl) were spiked into the samples during RNA isolation. Sequences of the applied quantitative PCR primers are listed in Supplemental Table S6.1 (supporting information).

Overall survival and recurrence-free survival

Overall survival was calculated from the date of partial hepatic resection to the date of decease or to the date of last follow-up when patients were not deceased. Recurrence-free survival was calculated from the date of partial hepatic resection to 1) the date of first radiological examination with diagnosis of a recurrent lesion or 2) the date of last radiological follow-up when recurrence had not occurred. Assessment of recurrence was based on computed tomography, positron emission tomography-computed tomography examinations, or magnetic resonance imaging, and combined with pathology reports or carcinoembryonic antigen test whenever available. Overall recurrence included not only liver recurrence, but also lung, primary colorectal site, and other types of recurrence.

Statistical analyses

We considered microRNAs differentially expressed when they fulfilled the combination of the following criteria: 1, a p value < 0.05 determined by an unpaired moderated T -test using the Bioconductor's Limma package;¹⁹ 2, absolute fold change (FC) >1.2 (in accordance with $\log_2$ ratio of <-0.263 or >0.263).

All other statistical analyses were conducted using IBM SPSS software for Windows (version 20.0). For continuous variables p -values were calculated using Mann-Whitney U test; and for categorical variables, Chi-square test with continuity correction or Fisher's exact test was used. Spearman correlation analysis was used to assess the correlation between parameters of interest. Survival curves were generated according to the Kaplan-Meier method in GraphPad Prism 6 for Windows (Version 6.05), and the p values were computed using the Log-rank test. The median follow-up was calculated according to the reverse Kaplan-Meier technique. A p value of less than 0.05 was considered statistically significant for all tests.

Results

Patient characteristics

Of the 40 patients assessed by pathologists, 28 displayed SD grade 0, 2, or 3 and were finally included in this study (SD grade 2-3 *versus* grade 0). General characteristics of these patients are summarized in Table 6.1. Of the 28 patients included, 20 patients could be matched for important parameters such as oxaliplatin exposure, sex, age, BMI, and liver injury markers, and were selected for subsequent microarray analyses. Group 1 ($n=10$) consisted of patients with grade 2-3 SD. Group 2 ($n=10$) consisted of patients without SD (grade 0). General characteristics of these patients are summarized in Table 6.2.

Reduced miR-21 and miR-150 expression levels in the liver of patients with severe SD

To investigate which microRNAs were differentially expressed in the liver of patients with and without SD, we performed microarray analysis using 20 prudently matched specimens. Surprisingly, not a single microRNA was significantly upregulated in the livers of patients with grade 2-3 SD as compared to patients without SD. Moreover, only two microRNAs were significantly downregulated in patients with SD grades 2-3 (Figure 6.1, A and B): miR-21 and miR-150. Hepatic miR-21 expression was 1.34-fold lower in patients with SD ($p=0.028$), whereas miR-150 expression was reduced to a larger extent in this population (1.46-fold, $p=0.035$).

Table 6.1 General characteristics of all included patients.

	Sinusoidal Dilatation Absent (n=14)	Sinusoidal Dilatation Grade 2-3 (n=14)	p value
<i>General characteristics</i>			
Sex (male)	6	6	1.000
Age (years)	64±3	60±3	0.329
BMI (kg/m ²)	25.6±1.0	25.5±0.8	0.910
ALT (U/L)	37±4	29±4	0.219
AST (U/L)	32±4	26±4	0.410
<i>Chemotherapy</i>			
Number of Oxaliplatin cycles	6.1±0.6	5.7±0.6	0.667
Cumulative Oxaliplatin dose (mg)	1345±121	1362±128	1.000
Bevacizumab treatment	11	10	1.000
<i>Other characteristics</i>			
Major hepatectomy	5	6	1.000
Perioperative blood transfusion (yes)	7	3	0.236
Morbidity			
None	6	7	1.000
Minor (Clavien-Dindo grade <3a)	3	5	0.678
Major (Clavien-Dindo grade ≥3a)	5	2	0.385
Length of hospital stay (days)	12±3	12±4	0.756

SD, sinusoidal dilatation; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; continuous variables are presented as mean (standard deviation), and *p*-values were calculated using Mann-Whitney U test; categorical variable was tested using Pearson Chi-Square test with continuity correction.

Table 6.2 General characteristics of patients matched for microarray analyses.

	Sinusoidal dilatation		p-value
	Absent (n=10)	Grade 2-3 (n=10)	
Sex (male)	5	5	1.000
Age (years)	61.9 (9.3)	58.2 (13.6)	0.481
Body mass index (kg/m ²)	26.1 (4.3)	26.4 (2.9)	0.853
Alanine aminotransferase (U/L)	33.3 (13.8)	30.1 (15.0)	0.604
Aspartate aminotransferase (U/L)	28.1 (15.2)	27.7 (13.4)	1.000
Number of oxaliplatin cycles	6.4 (2.6)	6.3 (2.2)	1.000
Cumulative oxaliplatin dose (mg)	1393 (468)	1496 (442)	0.796

Continuous variables are presented as mean (standard deviation), and *p*-values were calculated using Mann-Whitney U test; categorical variable was tested using Pearson Chi-Square test with continuity correction.

To validate these microarray data, we first performed quantitative PCR using liver specimens from the same set of patients. Again, miR-21 and miR-150 were found to be downregulated in the liver of patients with grade 2-3 SD, confirming the array data. The fold change of miR-21 was 1.34 (*p*=0.015); the fold change of miR-150 was 1.39 (*p*=0.011). Next, we further validated hepatic downregulation of miR-21 and miR-150 in grade 2-3 SD using the entire set of patients with and without SD who fulfilled the

inclusion criteria (n=28). In line with the microarray data, we found a significant downregulation of both miR-21 and miR-150 in patients with grade 2-3 SD. The fold changes were similar to those observed in the microarray experiment (1.32, $p=0.001$ and 1.34, $p=0.014$, respectively (Figure 6.1, C and D)).

Figure 6.1 Reduced relative hepatic miR-21 and miR-150 expression in high grade sinusoidal dilatation patients.

A and B: Compared with the non- sinusoidal dilatation group, both hepatic miR-21 (**A**) and miR-150 (**B**) were downregulated in the severe sinusoidal dilatation group as assessed by microarray analysis (n=20). **C and D:** Quantitative PCR analysis of a slightly bigger set of patients (n=28)

validated downregulation of miR-21 (C) and miR-150 (D) in severe sinusoidal dilatation.

Hepatic microRNA expression changes in SD are not reflected in plasma

There is an increasing interest in the use of circulating microRNAs as biomarkers of disease. In view of the downregulation of miR-21 and miR-150 in the liver of patients with grade 2-3 SD, we next explored the possibility that these microRNA changes would also be detectable in plasma, offering a way to monitor SD development and/or progression during chemotherapy treatment. Both miR-21 and miR-150 were detectable in the plasma of patients either with or without SD. However, there were no significant differences in circulating levels of microRNA between these patients groups (Figure 6.2, A and B). Moreover, whereas hepatic and plasma levels of miR-150 were correlated, this was not found for miR-21 (Figure 6.2, C and D).

Low hepatic miR-21 and miR-150 expression is related to impaired survival

MiR-21 and miR-150 have been demonstrated to play important roles in tumour cell proliferation, migration, and invasion, thereby affecting postoperative survival in colorectal cancer patients.^{12,20} In addition, patients with SD are known to display earlier recurrence and shorter overall survival after partial hepatectomy for colorectal liver metastases.⁹ We therefore next investigated the potential link between SD, miR-21 and miR-150 expression, and survival.

The median follow-up was 83 months for overall survival, 66 months for overall recurrence-free survival, and 61 months for liver recurrence-free survival. Of the 28 patients studied, 20 patients developed recurrence within the follow-up period; 1 patient underwent non-radical hepatic resection and was excluded from this analysis. The recurrence sites were liver (n=9), lung (n=5), skeleton (n=3), primary colorectal site (n=1), mediastinum (n=1), and peritoneum (n=1).

Surprisingly, SD was neither associated with impaired overall survival ($p=0.711$) nor with overall recurrence-free survival ($p=0.900$) or liver recurrence-free survival ($p=0.971$) in our patient group. However, when we defined 50th percentile groups of high and low microRNA expression, we found that low expression of liver miR-21 was related to impaired overall survival ($p=0.032$, Figure 6.3A) as well as shorter overall recurrence-free survival ($p=0.010$, Figure 6.3B) and shorter liver recurrence-free survival ($p=0.046$, Figure 6.3C). Similarly, low hepatic miR-150 expression was related to impaired overall survival ($p=0.010$, Figure 6.3D), and reduced overall recurrence-free survival ($p=0.025$, Figure

6.3E) as well as shorter liver recurrence-free survival ($p=0.031$, Figure 6.3F). There was no correlation between miR-21 and miR-150 in plasma and overall survival or recurrence-free survival (data not shown).

Figure 6.2 Hepatic microRNA expression changes in sinusoidal dilatation are not reflected in plasma.

A and B: Similar plasma miR-21 (**A**) and miR-150 (**B**) concentrations in patients with and without sinusoidal dilatation ($n=27$), $p=0.867$ and $p=0.519$, respectively. **C:** Hepatic miR-21 expression was not correlated with miR-21 plasma levels, $r=0.081$, $p=0.687$. **D:** Correlation between hepatic miR-150 expression and miR-150 plasma levels, $r=0.438$, $p=0.022$.

Figure 6.3 Low hepatic miR-21 and miR-150 expression are correlated with impaired long-term survival. **A:** Low hepatic miR-21 expression is associated with reduced overall survival. **B:** Patients with low hepatic miR-21 expression display reduced overall recurrence-free survival. **C:** Reduced liver recurrence-free survival in patients with low hepatic miR-21 expression. **D:** Low hepatic miR-150 expression is related to reduced overall survival. **E:** Patients with low hepatic miR-150 expression display reduced overall recurrence-free survival. **F:** Patients with low hepatic miR-150 expression display reduced liver recurrence-free survival.

Discussion

SD is a serious complication in patients with CRLM treated with oxaliplatin-based chemotherapy. It impairs long term survival and negatively affects tumour response to chemotherapy treatment.^{6,7} The pathophysiological mechanisms responsible for the development of SD as well as its detrimental effects on patient outcome are still unclear. In the present study, we show that SD is characterized by reduced hepatic miR-21 and miR-150 expression, and that low expression of these microRNAs is associated with poorer survival.

Previous studies have implicated miR-150 in the context of colorectal cancer. For example, miR-150 expression in colorectal tissue was recently shown to diminish in parallel with increasing carcinogenesis.¹² In line with our data, low miR-150 expression was associated with poor survival as well as a reduced response to adjuvant chemotherapy.¹² Interestingly, whereas colorectal tumour displayed lower miR-150 expression than normal colorectal mucosa,¹¹ patients with colorectal tumour with liver metastases had higher tumour miR-150 expression than those without liver metastases.²¹ Together with the fact that miR-150 is also expressed in other tissues such as adipose tissue,²² this may explain why we did not find reduced miR-150 levels in the plasma of patients with SD, who were characterized by lower hepatic miR-150 expression.

A key question is how reduced miR-150 expression may contribute to the development of SD, and how this might impact survival. With respect to survival, it is noteworthy that miR-150 has been suggested to function as tumour suppressor in colorectal cancer. For example, miR-150 appears to inhibit colorectal tumour cell migration and invasion by targeting MUC4.²³ Furthermore, miR-150 has been suggested to induce colorectal tumour cell apoptosis and to reduce cell migration and invasion by inhibiting c-Myb, which is overexpressed in colon tumour and plays crucial roles in cell proliferation.²⁰ In addition, miR-150 targets vascular endothelial growth factor (VEGF),²⁴ a factor that is known to induce endothelial cell migration and mediate the formation of new blood vessels in tumour. Since VEGF has been found to be upregulated in oxaliplatin-related severe SD,¹⁰ the effect of miR-150 on VEGF may also be relevant in the context of SD. SD-associated VEGF upregulation is consistent with the downregulation of miR-150 that we observed in the liver of patients with SD, and suggests that low miR-150 levels may contribute to the observed increase in VEGF in SD. VEGF upregulation, in turn, has been suggested to increase the expression of matrix metalloproteinases that promote detachment of sinusoidal endothelial cells from the basement membrane and promote

SD development.¹⁰ An additional potential mechanism by which miR-150 may affect SD relates to its suppressive effect on hepatic stellate cells activation.²⁵ Hepatic stellate cells promote SD progression by depositing high amounts of collagen in the space of Disse's,^{5,10} a process that appears to be suppressed by the binding of miR-150 to collagen 4A4 and Sp1, a mediator of α -1(I) collagen expression.²⁵ Thus, taken together, miR-150 downregulation may play a crucial role not only in promoting the development of SD but also in reducing tumour suppression, thereby potentially explaining the relation between miR-150 expression and impaired overall and recurrence-free survival that we observed.

In contrast to the reduced expression of miR-150 in colorectal cancer, miR-21 appears to be overexpressed in colorectal cancer cells.²⁶ In addition, higher serum and plasma miR-21 concentrations have been reported in colorectal cancer patients,¹⁵ and high colorectal tumour miR-21 expression has been associated with poorer prognosis in colorectal cancer patients.²⁷ On the other hand, it was recently shown that reduced serum miR-21 levels are related to higher local colorectal cancer recurrence.¹⁴ This is in line with the decreased overall and recurrence-free survival that we observed in patients with low hepatic miR-21 expression.

Mechanistically, antisense inhibition of miR-21 has been shown to inhibit tumour cell migration and to sensitize tumour cells to anticancer agents, indicating that miR-21 plays an active role in colorectal tumour progression.²⁸ Furthermore, miR-21 overexpression has been previously demonstrated to impair angiogenesis from endothelial cells by inhibiting RhoB.²⁹ Importantly, gene expression profiling revealed that the angiogenesis pathway is upregulated in oxaliplatin-related SD.¹⁰ Thus, the low miR-21 expression we found in patients with SD may contribute to the increased expression of proteins related to angiogenesis. Unlike miR-150, miR-21 activates hepatic stellate cells via PTEN/Akt signaling, and stimulates matrix metalloproteinases.³⁰ The reduced expression of miR-21 in severe SD may therefore partly balance the detrimental effects of miR-150 on hepatic stellate cell activation and collagen deposition.

The fact that our study could not confirm the previously reported⁹ association between SD and impaired survival may be due to selection bias in our relatively small study cohort. Indeed, we mainly controlled for factors that we expected to affect gene expression, but that are not necessarily related to prognostic factors of survival.

To our knowledge, this is the first study to investigate the microRNA expression profile of oxaliplatin-related SD. Both identified downregulated microRNAs may not only play

important roles in SD development, but also impact overall survival. Further studies focused on the genes targeted by miR-21 and miR-150 will likely contribute to revealing the mechanisms of oxaliplatin-related SD and could support clinicians to inhibit the development of SD, improving postoperative outcomes.

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Supplemental tables

Table S6.1 Primers for quantitative PCR.

Species	Name of primer	Sequence
Human	Human miR-150 forward	5' - TCTCCCAACCCTTGACCACTG
	Human miR-21 forward	5' - TAGCTTATCAGACTGATGTTGA
	Human miR-16 forward	5' - TAGCAGCACGTAAATATTGGCG
Caenorhabditis elegans	Cel-miR-39 forward	5' - TCACCGGGTGTAATCAGCTTG
	Cel-miR-54 forward	5' - TACCCGTAATCTTCATAATCCGAG
	Cel-miR-238 forward	5' - TTTGTACTCCGATGCCATTCAGA

Chapter 7

Hepatic complement activation is associated with liver injury in patients with colorectal liver metastases

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Submitted

Abstract

Background

Preoperative systemic chemotherapeutic treatment for colorectal liver metastases frequently induces chemotherapy-associated liver injury (CALI). CALI encompasses liver phenotypes such as sinusoidal dilatation, nodular transformation, hepatocyte steatosis, and lobular inflammation. Since complement factors are known to be involved in these hepatic pathophysiologies, we investigated the link between complement activation and CALI.

Methods

50 consecutive patients who underwent partial hepatectomy for colorectal liver metastases (CRLM) were included. Sinusoidal dilatation and nodularity were assessed using formalin-fixed non-tumour liver samples. C3c, C5a, and terminal complement complex (TCC) concentrations were determined in liver tissue lysates by ELISA to investigate complement activation; neutrophil infiltration was assessed by myeloperoxidase staining and subsequently quantified as a measure of lobular inflammation; hepatic triglyceride concentration was analyzed to quantify steatosis.

Results

Hepatic C3c and C5a were both detectable, and C3c concentrations strongly correlated with C5a concentrations ($r=0.684$, $p<0.0001$). C3c concentration was similar in patients with or without sinusoidal dilatation (13.6 ± 1.6 vs. 13.5 ± 1.2 ng/ml, $p=0.952$) and in patients with or without nodular transformation (14.2 ± 3.2 vs. 13.4 ± 1.1 ng/ml, $p=0.750$). In contrast, hepatic C5a levels were increased in patients with nodular transformation (13.9 ± 4.3 ng/ml vs. 7.4 ± 1.1 ng/ml, $p=0.041$), but not in those with sinusoidal dilatation (10.4 ± 2.1 ng/ml vs. 6.7 ± 0.9 ng/ml, $p=0.220$). Hepatic concentrations of C3c and C5a correlated with hepatic neutrophil infiltration ($r=0.373$, $p=0.008$; $r=0.485$, $p=0.0004$, respectively), but not with triglyceride concentrations.

Conclusions

The complement system appears to be activated in the livers of CLRM patients with hepatic nodular transformation and lobular inflammation. This warrants investigation of the potential effect of complement inhibition on the development of CALI.

Introduction

Over the past decades, patients with colorectal liver metastases (CRLM) have been commonly treated with combined chemotherapeutic agents such as irinotecan and oxaliplatin. This strategy increased tumour response rates considerably from 24% to up to 70%, compared to single treatment with 5-fluorouracil.^{1,2} However, neoadjuvant administration of these agents frequently has harmful side-effects, referred to as chemotherapy-associated liver injury (CALI). For instance, oxaliplatin-based treatments increase the occurrence of sinusoidal dilatation³ and the risk of nodular regenerative hyperplasia (NRH).³⁻⁵ Irinotecan-based regimens appear to be related to the development of steatohepatitis.⁶

Pathophysiological processes leading to CALI include the activation of the acute phase response,⁷ which is characterized by increased secretion of complement factors by the liver. As a pivotal element of the innate immune system, the complement system mediates the initiation of inflammatory reactions, the clearance of immune complexes, and the regulation of the immune response to tissue injury.⁸ Activation of the central complement factor C3 results in the formation of C3a, a chemokine that recruits neutrophils into the liver and participates in the development of nonalcoholic steatohepatitis.⁹ Next, C3a is rapidly cleaved by carboxypeptidases to generate acylation stimulating protein (ASP), a peptide which plays a role in triglyceride metabolism and has been shown to be increased in nonalcoholic steatohepatitis.^{10,11}

Sustained activation of C3 can result in the formation of C5 convertase, which cleaves complement factor C5 into C5b and C5a. Similar to C3a, C5a also promotes inflammation by attracting neutrophils.¹² Moreover, it has recently been shown that C5a stimulates hepatic stellate cells and plays a causal role in the regulation of liver fibrosis in various liver diseases.¹³⁻¹⁵ In addition, C5a has been shown to play a role in the early stages of liver regeneration after partial hepatectomy.^{16,17}

Given the involvement of complement factors in these pathophysiological processes that also occur in CALI, we hypothesized that complement activation could play a role in patients undergoing partial hepatectomy for CRLM.

Materials and methods

Patients and sample selection

Consecutive patients who underwent partial hepatectomy for CRLM at the Maastricht University Medical Centre between January 2008 and December 2009 were included in this study. Exclusion criteria were: a) patients who underwent preoperative portal venous embolization, since this procedure induces hepatic ischemia which probably interacts with sinusoidal changes related to chemotherapy; b) patients who were diagnosed with autoimmune liver disease, type B or C hepatic virus infection; c) patients with cirrhosis. Non-tumour liver specimens at a distance of more than 2 cm from the tumour were collected by two approaches: a) samples collected after opening the abdomen and before touching or manipulating the intestines or liver to prevent artificial influx of inflammatory cells¹⁸ were immediately snap-frozen in liquid nitrogen and stored at -80°C; b) Once the tumour was resected, additional non-tumour liver samples (at a distance of more than 2 cm from the tumour) were collected, fixed in 4% buffered formaldehyde, and embedded in paraffin. Frozen samples were used to quantify C3c, C5a, TCC, and triglyceride concentrations, and to stain neutrophils. Formalin-fixed paraffin-embedded samples were used to assess liver injuries (i.e. sinusoidal dilatation and nodular transformation). This study was approved by the Medical Ethics Committee of Maastricht University Medical Centre and conducted according to the revised version of the Declaration of Helsinki. Written informed consent was obtained from each subject prior to study participation.

Assessment of sinusoidal dilatation and nodular transformation

Morphological assessment was performed based on hematoxylin and eosin (H&E), Masson's Trichrome, and reticulin stains as previously described.¹⁹ All specimens were independently reviewed by two experienced liver pathologists (C.V. and A.D.) to assess sinusoidal dilatation and nodular transformation in non-tumour liver using paraffin embedded tissue. Severity of sinusoidal dilatation was semi-quantified according to the classification of Rubbia-Brandt et al. as follows: 0, absent; 1, mild (centrilobular involvement limited to one-third of the lobular area); 2, moderate (centrilobular involvement extending in two-thirds of the lobular area); 3, severe (complete lobular involvement or centrilobular involvement extending to adjacent lobules with bridging congestion).³ Nodular transformation was assessed according to Wanless as follows: 0, absent; 1, mild (focal occasionally distinct nodular hyperplasia on reticulin staining but indistinct on H&E staining); 2, moderate (focal distinct nodular hyperplasia apparent on H&E staining, clearly highlighted on reticulin staining); 3, severe (diffuse nodular

hyperplasia, distinct in most areas on H&E staining and highlighted on reticulin staining, corresponding to nodular regenerative hyperplasia).²⁰ Afterwards, patients were divided into two groups: those without liver injury (absent) *versus* those with liver injury (mild, moderate, or severe).

Quantification of hepatic C3c, C5a, and TCC concentration

Liver tissue lysates were made by homogenizing frozen liver tissue in ice-cold lysis buffer (pH 7.4), which contained 30 mM Tris-HCl, 150 mM NaCl, 0.5% Triton X-100, 1% (v/v) SDS, 100 mM EDTA, 10 mM NaF, and 1 mM Na₃VO₄. Hepatic concentrations of C3c, C5a, and terminal complement complex (TCC) were measured using human C3c, C5a, and TCC ELISA kits (Hycult Biotech, Uden, NL) according to the manufacturer's protocol. The final concentration was determined by referencing to the total protein concentration in the lysate as determined by the bicinchoninic acid (BCA) protein assay kit (ThermoFisher Scientific, Landsmeer, NL) according to the manufacturer's protocol.

Quantification of liver triglyceride levels

To quantify liver fat content, we measured liver triglyceride concentrations in tissue lysates using the triglyceride colorimetric assay kit (Cayman chemical, Uden, NL). The procedures were performed according to the manufacturer's protocol. The final concentration of liver triglyceride was determined by referencing to the total protein concentration.

Immunohistochemistry

Immunohistochemical staining was performed as previously described.²⁰ Un-manipulated frozen non-tumour liver tissue was cut into 4- μ m-thick sections. Endogenous peroxidase activity was blocked by incubation in 0.6% H₂O₂ for 15 minutes. After blocking with 5% bovine serum albumin, primary polyclonal rabbit antibody recognizing human myeloperoxidase (dilution 1:500; Dako, Heverlee, Belgium) was applied for 1 hour. Biotinylated swine-anti-rabbit IgG (dilution 1:500; Dako) was applied as secondary antibody for 1 hour, and followed by application of avidin-biotin-horseradish peroxidase system (dilution 1:50; DAKO). 3-Amino-9-ethylcarbazole was used as chromogen. Tissue sections were counterstained with Mayer's hematoxylin. Neutrophil numbers were quantified by photographing five randomly selected parts of myeloperoxidase-stained sections at 100 x magnifications followed by manual counting of stained cells.

Statistical analysis

All statistical analyses were conducted using Graphpad Prism for Windows (version 6.05). The Student's t-test was used for continuous variables. For categorical variables, the Pearson Chi-square test with continuity correction was applied, or the Fisher's exact test when any of the expected values was smaller than five. Pearson product-moment correlation coefficient was computed and scatterplots were drawn to assess the relationship between two parameters of interest. A p value ≤ 0.05 was considered significant for all analyses.

Results

Patient characteristics

A total of 50 patients were included. Oxaliplatin, in combination with or without bevacizumab, was the most commonly administered chemotherapeutic agent (41/50). Details of the applied chemotherapy and general characteristics of the patients are reported in Table 7.1. Pathology examination of the non-tumour tissue revealed that 32 (64%) patients had sinusoidal dilatation, and 13 (26%) patients displayed nodular transformation. Patients with sinusoidal dilatation showed a higher prevalence of nodular transformation than those without sinusoidal dilatation (12/32 vs. 1/18, $p=0.024$).

Table 7.1 General characteristics of included patients.

	Sinusoidal dilatation			Nodular transformation		
	None (n=18)	Present (n=32)	p	None (n=37)	Present (n=13)	p
Sex (male)	9 (50%)	18 (56%)	0.897 [†]	19 (51%)	8 (62%)	0.756 [†]
Age (years)	65 (9)	62 (11)	0.497	63 (10)	63 (12)	0.986
BMI (kg/m ²)	26.3 (3.7)	25.8 (3.1)	0.606	25.7 (3.4)	26.9 (3.0)	0.241
AST (U/L)	29 (14)	25 (11)	0.235	26 (13)	28 (11)	0.611
ALT (U/L)	33 (15)	27 (11)	0.107	30 (13)	27 (13)	0.531
Oxaliplatin	14 (78%)	25 (78%)	1.000 [‡]	28 (76%)	11 (85%)	0.808 [‡]
Oxaliplatin cycles	4.8 (3.4)	4.2 (2.9)	0.493	4.0 (3.0)	5.5 (3.1)	0.137
Irinotecan	1 (6%)	4 (13%)	0.802 [‡]	3 (8%)	2 (15%)	0.778 [‡]
Irinotecan cycles	0.3 (1.4)	0.6 (1.9)	0.614	0.6 (2.0)	0.3 (0.9)	0.646
Capecitabine	14 (78%)	27 (84%)	0.826 [‡]	30 (81%)	11 (85%)	1.000 [‡]
Capecitabine cycles	4.6 (3.0)	4.8 (3.2)	0.755	4.3 (2.7)	6.1 (3.8)	0.069
Bevacizumab	10 (56%)	20 (63%)	0.857 [†]	22 (60%)	8 (62%)	1.000 [†]
Nodular transformation (present)	1 (6%)	12 (38%)	0.024 [‡]	n.a.	n.a.	n.a.

BMI, body mass index; AST, aspartate transaminase; ALT, alanine transaminase; n.a., not applicable; continuous data are shown as mean (standard deviation), and tested by Student's t test; [†]Pearson Chi-square test, with continuity correction; [‡]Fisher's exact test

Complement activation in the liver of patients with CRLM

C3c has been shown to be a stable complement activation biomarker that is generated through cleavage of C3.²² Hepatic C3c and C5a were detectable in all patients, indicating there was complement activation in the non-tumour liver parenchyma. The mean concentrations of C3c and C5a were 13.6 ng/ml and 9.1 ng/ml, respectively. As expected, C3c and C5a concentrations showed a strong, positive correlation ($r=0.684$, $p<0.0001$, Figure 7.1), indicative of sustained complement activation. However, hepatic TCC concentrations were too low to be detected.

Figure 7.1 Correlation between hepatic C3c and C5a concentration. Pearson correlation analyses showed a positive correlation between hepatic concentrations of C3c and C5a ($r=0.684$, $n=50$, $p<0.0001$)

Increased hepatic C5a levels in patients with nodular transformation

Considering that oxaliplatin treatment is a risk factor for sinusoidal dilatation and nodular transformation,^{3,5} pathophysiologic processes underlying these hepatic parenchymal changes might be similar. Therefore, we next investigated a potential relationship between complement activation and these pathologies by comparing hepatic C3c and C5a concentrations in patients with and without sinusoidal dilatation or nodular transformation.

Neither C3c levels (13.6 ± 1.6 ng/ml vs. 13.5 ± 1.2 ng/ml, $p=0.952$; Figure 7.2A) nor concentrations of C5a (10.4 ± 2.1 ng/ml vs. 6.7 ± 0.9 ng/ml, $p=0.220$; Figure 7.2B) were significantly different between patients with and without sinusoidal dilatation. Although C3c levels were not significantly different between patients with and without nodular transformation (14.2 ± 3.2 ng/ml vs. 13.4 ± 1.1 ng/ml, $p=0.750$; Figure 7.2C), C5a

concentrations were increased in patients with these lesions (13.9 ± 4.3 ng/ml vs. 7.4 ± 1.1 ng/ml, $p=0.041$; Figure 7.2D).

Figure 7.2 Hepatic C5a levels are not significantly increased in patients with sinusoidal dilatation, but increased in those with nodular transformation.

A and B: Hepatic C3c (**A**) and C5a (**B**) concentrations are not significantly different in patients with and without sinusoidal dilatation, $p=0.952$ and $p=0.220$, respectively. **C:** Similar hepatic C3c concentration in patients with and without nodular transformation, $p=0.750$. **D:** Increased hepatic C5a concentrations in patients with nodular transformation, $p=0.041$. SD, sinusoidal dilatation; Error bar represents mean with standard error of mean

Hepatic C3c and C5a concentrations correlate with hepatic neutrophil infiltration

Next, we investigated potential links between complement activation and hepatic steatosis as well as inflammation, two important elements of CALI. Hepatic triglyceride

concentrations were not correlated with either hepatic C3c ($r=0.070$, $p=0.627$; Figure 7.3A) or C5a levels ($r=-0.173$, $p=0.229$; Figure 7.3B). In contrast, correlations were found between hepatic C3c concentration and neutrophil infiltration ($r=0.373$, $p=0.008$; Figure 7.3C), as well as between hepatic C5a concentration and neutrophil infiltration ($r=0.485$, $p=0.0004$; Figure 7.3D). These data suggest that complement activation in subjects with CALI is related to hepatic inflammation independent of steatosis.

Figure 7.3 Hepatic concentrations of C3c and C5a are correlated with liver inflammation but not with steatosis.

A and B: Hepatic triglyceride concentration was not correlated with liver C3c (**A**) and C5a (**B**) levels, $r=0.070$, $p=0.627$; $r=-0.173$, $p=0.229$, respectively. **C:** Significant correlation between hepatic C3c concentrations and neutrophil infiltration, $r=0.373$, $p=0.008$. **D:** Highly significant correlation between hepatic C5a concentration and neutrophil infiltration, $r=0.485$, $p=0.0004$.

Discussion

This study has shown that complement activation occurs in the non-tumour liver of patients with CRLM. Hepatic C5 activation was specifically increased in patients with nodular transformation and tended to be increased in patients with sinusoidal dilatation. Hepatic inflammation as reflected by neutrophil infiltration was markedly higher in patients with hepatic C3 and C5 activation. Steatosis was neither associated with activation of C3 nor with activation of C5.

The link between hepatic inflammation and complement activation has previously been shown in other liver diseases, including nonalcoholic and alcoholic fatty liver disease, viral hepatitis, and hepatic ischemia/reperfusion.^{8,21,23,24} It is likely related to the generation of C3a and C5a, small complement peptides that have a strong chemotactic activity towards neutrophils and other leukocytes.²⁵ These complement peptides are formed when C3 and C5 are activated through cleavage by C3 and C5 convertases. Activation of these complement factors may be initiated through the so-called classical pathway, the lectin pathway, and/or the alternative pathway.²⁶ In the context of CALI, the induction of apoptosis in hepatocytes²⁷ may drive complement activation through the classical pathway²⁸. Apoptotic bodies bind to the complement component C1q which assists in their clearance after subsequent activation of C3 and binding of C3b. C3b is then further metabolized generating C3c, one of the complement factors that we found to be detectable in the non-tumour liver of patients with CRLM.

It has previously been suggested that the alternative pathway of complement might be involved in sinusoidal obstruction syndrome.²⁹ Patients with sinusoidal obstruction syndrome secondary to receiving a hematopoietic stem cell transplantation and cyclophosphamide treatment for acute myeloid leukaemia were found to have single nucleotide polymorphisms in the gene encoding the complement regulatory protein factor H.²⁹ These polymorphisms had previously been associated with atypical haemolytic uremic syndrome, a complement-driven disease.³⁰ Complement activation was not significantly increased in subjects with sinusoidal dilatation in the present study, although we did observe a trend towards higher hepatic C5a levels in these patients. This could be related to the fact that the causes of sinusoidal dilatation in hematopoietic stem cell transplantation are different from those in sinusoidal dilatation. Hence, mechanisms underlying this type of liver injury are likely to be different as well.

Complement factors have also been shown to be involved in liver regeneration. In particular, C5 appears to be important given the severely compromised regenerative

response after acute toxic liver injury in C5-deficient mice and the abrogation of hepatocyte proliferation after blockage of the C5a receptor.¹⁶ By stimulating the C5a receptor on the surface of Kupffer cells, C5a can modulate the release of IL-6 and TNF- α , which play a role in the initiation of hepatocyte proliferation.¹⁷ This important role of C5a in liver regeneration is supported by our data showing that C5a was elevated in patients with nodular transformation, which is considered to be a regenerative response to sinusoidal injury.²⁰

It remains to be determined why TCC was not detectable in the liver even though C3 and C5 activation did occur. Formation of TCC is the ultimate result of complement activation, and TCC has a direct lytic capacity to kill injured cells.²⁶ Hence, TCC activation is firmly regulated to avoid accidental cell damage. The lytic part of TCC can be removed within 5-10 minutes after deposition on the membrane of target cells through in-/outward vesiculation.³¹ In addition, the TCC component C8 can act as a TCC inhibitor.³² Furthermore, CD59, expressed on hepatocytes, negatively regulates TCC via interaction with the other TCC subunits, C5b-C9.^{12,26} Taken together, both the short half-life of TCC and its tight negative regulation might be accountable for the lack of detectable hepatic TCC in our tissue samples.

As a final remark, it should be mentioned that we analysed a relatively small number of patients. Therefore, the link between complement activation and hepatic nodularity needs to be validated by future research. Nevertheless, our data do provide the first evidence that various aspects of CALI are associated with complement activation. In view of this, investigation of the potential impact of complement inhibition using existing drugs³³⁻³⁵ on the development of CALI is warranted.

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Chapter 8

Summary, implications, and future perspective

A large body of literature has been published demonstrating the benefits of partial hepatectomy for increasing long-term survival in patients with colorectal liver metastases (CRLM).¹ Surgery remains the mainstay of the CRLM treatment algorithm and provides the best prospect of cure.^{2,3} To further increase the cure rate, surgeons have developed many advanced surgical procedures for tumours traditionally considered irresectable, particularly multiple tumours with bilobular distribution. These procedures include two-stage hepatectomy,⁴ ultrasound-guided enhanced one-stage hepatectomy,⁵ and associating liver partition and portal vein ligation for staged hepatectomy (ALPPS).^{6,7} Apart from these advanced surgical techniques, neoadjuvant chemotherapy has been put forward as a treatment option that could reduce the tumour burden, thereby enabling surgical resection.^{1,8,9}

Surgical safety, expressed as incidence of postoperative morbidity and mortality, is of great importance in the context of partial hepatectomy, and has been reported to be negatively influenced by neoadjuvant chemotherapy.¹⁰ In particular, the presence of chemotherapy-associated liver injury (CALI) has been shown to impair liver regeneration, which is pivotal for recovery of liver function in patients after partial hepatectomy.¹¹ Moreover, CALI appears to be associated with reduced tumour responsiveness to neoadjuvant chemotherapy,^{12,13} which has implications for long-term recurrence-free survival and overall survival. Understanding the relationship between the impact of CALI on postoperative short- and long-term outcome, and the mechanisms underlying CALI is of key importance for improving surgical outcomes of patients undergoing partial hepatectomy for CRLM.

The central aim of the current thesis was to investigate the influence of CALI on postoperative short- and long-term outcome after partial hepatectomy for CRLM. This was then translated into three main specific aims: **1)** to study the impact of CALI on short-term morbidity and mortality after partial hepatectomy for CRLM (**Chapter 2**, **Chapter 3**, and **Chapter 4**); **2)** to investigate the influence of CALI on long-term recurrence-free survival and overall survival after partial hepatectomy for CRLM (**Chapter 5**); and **3)** to provide better understanding of the mechanisms underlying CALI, the first step towards identification of potential biomarkers to predict CALI and of molecular targets to treat or prevent CALI (**Chapter 6** and **Chapter 7**).

Part I. Influence of CALI on short-term outcome after partial hepatectomy for CRLM

The first aim of this thesis was to assess the impact of CALI on short-term and long-term outcome after partial hepatectomy for CRLM. **Chapter 2** describes a systematic review with meta-analysis conducted and reported in compliance with the relevant PRISMA and MOOSE guidelines on this important topic.¹⁴⁻¹⁶ We focused on the influence of chemotherapy-associated sinusoidal dilatation on morbidity after partial hepatectomy in patients with CRLM, and found that sinusoidal dilatation was not associated with any type of short-term outcome (i.e. overall morbidity, liver failure, liver-related morbidity, and mortality) after partial hepatectomy. It should be emphasized that the individual studies included in our meta-analysis had a considerable risk of bias (with consideration of study participation, study attrition, prognostic factor measurement, outcome measurement, study confounding, and statistical analysis and reporting¹⁷) and comparatively low quality of outcome-specific evidence, leading to limited confidence in the overall conclusion that sinusoidal dilatation is not important for morbidity after partial hepatectomy for CRLM. Variations in study design and inclusion criteria, diverse definitions of a single endpoint, and varied endpoints under the category of morbidity were factors that negatively affected the quality of the evidence. In light of these concerns, no solid conclusions could be drawn and this study therefore cannot provide clinical advice on the topic.

Implications of Chapter 2

In the current era of evidence-based practice, meta-analyses or randomized controlled trials deliver the highest level of evidence. Conclusions drawn from these types of studies are therefore regularly incorporated into clinical guidelines. However, the definition, objectivity, and reproducibility of endpoints collected from studies performed in multiple centres determine whether multiple-origin data can be integrated and whether a robust conclusion can be drawn from a meta-analysis. Surgical outcome after partial hepatectomy must be standardized and defined in a way that enables clinicians to collect the data objectively and reproducibly. This will facilitate comparisons among multiple institutions, between different interventions, and within a single centre over time.¹⁸ **Chapter 2** emphasizes the critical importance of assessing the risk of bias and the evaluation of quality in meta-analyses, to provide the most robust level of evidence for clinical decision making. More importantly, it highlights the need for unambiguous definitions of outcome in surgical oncology.

In **Chapter 3**, the impact of chemotherapy-associated sinusoidal dilatation and additional major chemotherapy-associated non-tumour liver parenchymal damages (i.e. steatosis and steatohepatitis) on postoperative morbidity and mortality rates after partial hepatectomy was studied by performing a meta-analysis of individual participant data based on a systematic literature review. A comprehensive literature search was first performed following PRISMA and Moose guidelines to identify studies relevant to the aim.^{14, 15} Corresponding authors from each study that fulfilled the inclusion criteria were contacted subsequently for collaboration and for sharing original individual participant data of the published cohort. Next, aggregated data from each collaborator was re-constructed, pooled, and modelled simultaneously. A consolidate database comprising 788 patients who underwent partial hepatectomy for CRLM in eight centres was generated. The influence of preoperative chemotherapeutic agents on liver injury, and subsequently the effect of liver injury on short-term outcome after liver surgery were analysed applying one-step uni-/multi- variable binary logistic regression models.

We observed an increase in postoperative major morbidity (Dindo-Clavien grade III-V¹⁹) and liver surgery-specific complications (i.e. ascites, postoperative liver failure, bile leakage, intra-abdominal abscess, intra-abdominal haemorrhage, and operative mortality²⁰) after partial hepatectomy in patients with severe sinusoidal dilatation (grade 2-3) and steatohepatitis (non-alcoholic steatohepatitis score, NAS ≥ 4), respectively. Moreover, postoperative liver failure occurred more often in patients with severe sinusoidal dilatation. With respect to steatohepatitis, lobular inflammation, but not steatosis or hepatocellular ballooning, was strongly linked to increased postoperative morbidity. Oxaliplatin was a strong risk factor for severe sinusoidal dilatation in multivariable analysis, whereas the addition of bevacizumab was related to a twofold decrease in the occurrence of severe sinusoidal dilatation in patients who received oxaliplatin. We concluded that after partial hepatectomy, severe sinusoidal dilatation and steatohepatitis were related to increased postoperative major morbidity and liver surgery-specific complications. Moreover, postoperative liver failure occurred more often in patients with severe sinusoidal dilatation. Oxaliplatin was a strong risk factor for the development of severe sinusoidal dilatation, whereas the addition of bevacizumab to oxaliplatin-based regimen reduced the occurrence of severe sinusoidal dilatation.

Implications of Chapter 3

Individual participant data analysis offers many advantages. In particular, it reduces reliance on the reporting quality of individual studies. This allowed us to re-define outcome by applying a unified definition. Furthermore, we were now able to choose a consistent analysis approach. This resulted in the first study to demonstrate both the

effect of chemotherapy on liver injury and, subsequently, the effect of liver injury on short-term postoperative outcome in a large, multicentre patient cohort. In view of the developed measures to diagnose CALI preoperatively (e.g. by imaging modalities, histologic assessment, and biochemical analyses),²¹ and the negative relationship between CALI and postoperative morbidity, it is advised to adapt surgical management when CALI is confirmed, for instance by applying ablative therapy or two-stage hepatectomy. Moreover, with increased chemotherapy-related postoperative morbidity, decreased chemotherapy responsiveness,^{12, 13} and increasing doubts about the usefulness of neoadjuvant chemotherapy in certain patient groups,²² one could even speculate that some patients would benefit from immediate resection instead of neoadjuvant chemotherapeutical treatment.

Chapter 4 describes the impact of CALI on infectious complications after partial hepatectomy for CRLM. This topic could not be addressed in the systematic reviews described in chapters 2 and 3 since there are hardly any studies conducted on this topic. This chapter describes a study of 265 consecutive patients who had undergone partial hepatectomy for CRLM at Maastricht University Medical Centre. Data regarding postoperative infectious complications were prospectively monitored daily by an independent infection-control nurse. CALI, as reflected by sinusoidal dilatation, steatosis, and steatohepatitis was assessed by three experienced liver pathologists. We also monitored nodular regeneration, another type of liver injury that is known to be tightly associated with oxaliplatin-based treatment.²³ It is recently gaining attention as postoperative liver failure appears to occur more often when its presence is observed.²³ We observed that postoperative surgical site infections and systemic sepsis after partial hepatectomy for CRLM occurred more often in patients with hepatic steatosis, whereas neither sinusoidal dilatation nor nodular regeneration was related to infectious complications. Presence of severe surgical site infections (i.e. deep surgical site infections and intra-abdominal abscesses) was associated with increased 90-day mortality, prolonged length of hospital stay, decreased long-term recurrence-free survival, and reduced overall survival. In contrast, none of the studied expressions of CALI was related to survival. Whereas there was an inverse relation between severe steatosis and sinusoidal dilatation, nodular regeneration was positively correlated to the occurrence of sinusoidal dilatation. Irinotecan-based treatment and body mass index were associated with steatosis. We concluded that chemotherapy-associated steatosis was associated with increased infectious complications. The latter, in turn, was related to decreased recurrence-free survival and overall survival.

Implications of Chapter 4

Despite mechanisms underlying the negative relationship between postoperative surgical site infections and long-term survival remain unclear, overall body immunocompetence may play a crucial role, as supported by the following observations: surgical infections occur more often once the body immune system is suppressed.²⁴ Efforts to enhance the immune system may suppress tumour progression,²⁵ increase tumour response, and prolong long-term survival.²⁶ In essence, the presence of surgical site infections may reflect a relatively weaker innate or adaptive immunity. Tumour suppression may therefore be weakened, eventually resulting in more frequent tumour recurrence and decreased long-term overall survival.

Tumour response, which is expressed as tumour regression grade and represents the effect of chemotherapy on the tumour, has been shown to be positively associated with survival,¹³ whereas negatively related to the presence of chemotherapy-associated liver injury.¹² However, the influence of tumour regression grade on survival was not taken into account in this study. Therefore, future study is needed to clarify whether CALI itself or an impaired tumour response as a result of CALI is the key component associated with long-term recurrence-free survival and overall survival after partial hepatectomy for colorectal liver metastases. Given that postoperative infectious complications were found to be important prognostic factors for survival, such factors are advised to be included as confounders in future studies.

Steatohepatitis was previously determined by assessing the so-called nonalcoholic steatohepatitis activity score (NAS), which summarizes the severity of steatosis, lobular inflammation, and hepatocellular ballooning.²⁷ For the purpose of reducing inter-observer variations among pathologists, a novel scoring system (steatosis, activity, and fibrosis [SAF]) has been proposed in 2014,²⁸ and was applied to define steatohepatitis in this chapter. Surprisingly, application of the novel SAF scoring system was related to a very low incidence of steatohepatitis (0.4%) in our cohort. Although this low incidence could also be due to the fact that a minority of patients in the present cohort was treated with irinotecan, a known risk factor for steatohepatitis,²⁹⁻³¹ future studies assessing steatohepatitis by applying the SAF scoring system need to take this observed low incidence into account in the light of the number of patients to be included.

With respect to the adverse effect of hepatic steatosis on superficial surgical site infections and systemic sepsis, clinicians are advised to adopt measures for infection control when suspected hepatic steatosis is confirmed for instance through preoperative ultrasound or radiological images, or even seeing “yellow liver” during surgery.

Moreover, given that postoperative infectious complications were substantial prognostic factors for long-term survival, fundamental research dedicated to mechanisms underlying this observation are of interest. In addition, it is advised for future studies to consider postoperative infectious complications as potential confounders when identifying predictors for survival.

Chapter 5 summarizes a study which aimed to **1)** validate a potential negative influence of CALI on tumour responsiveness to therapy, and **2)** clarify the impact of CALI on long-term survival after partial hepatectomy for CRLM. In this study, we included 166 consecutive patients who were treated with neoadjuvant chemotherapy and underwent subsequent partial hepatectomy for CRLM. Since infectious complications were shown to be significant predictors of shortened long-term survival in **Chapter 4**, we specifically included them as confounder. Of all studied background liver abnormalities, only sinusoidal dilatation was related to diminished complete tumour response after neoadjuvant chemotherapy. As expected, complete tumour response was strongly associated with prolonged recurrence-free survival and overall survival. None of the background liver parenchymal abnormalities was associated with survival. Addition of bevacizumab chemotherapy was shown to decrease the incidence of sinusoidal dilatation and tended to be related to increased complete tumour response. We concluded that CALI was not related to survival. CALI was, however, associated with diminished complete tumour response, and tumour response, in turn, was associated with survival.

Implications of Chapter 5

Although sinusoidal dilatation was associated with diminished complete tumour response, and tumour response was associated with long-term survival, sinusoidal dilatation itself was not correlated with survival. Because neoadjuvant chemotherapy provides an increased complete tumour response³² which is related to prolonged survival, it could be speculated that the benefits of neoadjuvant chemotherapy in terms of survival outweigh the harms of inducing undesired parenchymal liver injury. In other words, chemotherapy could be more beneficial if clinicians could reduce the negative effects of CALI. Since addition of bevacizumab to neoadjuvant chemotherapy was associated with reduced sinusoidal dilatation and tended to be related to increased tumour response, it seems beneficial to add bevacizumab to oxaliplatin-based neoadjuvant chemotherapy.

Part II. Mechanisms underlying CALI

Previous chapters have demonstrated that CALI frequently occurred in patients who received chemotherapy, and that it was related to increased postoperative morbidity, and even compromised tumour responsiveness to neoadjuvant chemotherapy. Our next aim was therefore to provide a better understanding of the mechanisms underlying CALI, the first step towards identification of biomarkers for its preoperative diagnosis as well as therapeutic targets.

Chapter 6 summarizes a fundamental study which investigated the non-tumour hepatic microRNA expression profile in a setting of oxaliplatin-associated sinusoidal dilatation. In this study, we included a total of 28 patients who were treated with preoperative oxaliplatin-based chemotherapy and subsequently underwent partial hepatectomy for CRLM at Maastricht University Medical Centre. Ten patients with moderate to severe sinusoidal dilatation were matched with ten patients without sinusoidal dilatation by parameters which we suspected to affect microRNA expression (i.e. number of oxaliplatin cycles, cumulative oxaliplatin dose, sex, age, body mass index, and liver transaminases). MicroRNA expression was analyzed in the matched cohort through Agilent microarrays. Because the data were analysed without false discovery rate correction and there was potential bias caused by relatively small cohort size, a validation by qPCR was subsequently performed to confirm the findings from the microRNA array. We found that sinusoidal dilatation was not associated with upregulation of any microRNA, but was related to downregulation of miR-21 and miR-150. These findings were confirmed in a slightly larger Maastricht cohort of 28 patients. However, the downregulation of miR-21 and miR-150 in the liver of patients with sinusoidal dilatation was not found in plasma of the same patients. Given that miR-21 and miR-150 play important roles in tumour progression, affecting survival in colorectal cancer patients,^{33,34} and since sinusoidal dilatation compromises tumour response^{12,13} and may be further negatively related to survival,³⁵ we next explored the potential link between sinusoidal dilatation, miR-21 and miR-150 expression, tumour response, and survival. We found that sinusoidal dilatation was not associated with survival. However, low expression of miR-21 and miR-150 was related to impaired tumour response, and shortened recurrence-free as well as overall survival. We concluded that sinusoidal dilatation was not related to survival. Sinusoidal dilatation was, however, associated with expression levels of hepatic miR-21 and miR-150, and miR-21 and miR-150, in turn, were related to survival.

Implication of Chapter 6

Oxaliplatin-induced liver toxicity initially depletes glutathione and causes F-actin depolymerization, which results in rounding up and subsequent dehiscence of sinusoidal endothelial cells and obstruction of sinusoidal blood flow leading to sinusoidal dilatation.³⁶⁻³⁹ In line with these observations, gene expression of vascular endothelial growth factor (VEGF), a factor that is known to induce endothelial cell migration and mediate angiogenesis, is upregulated in oxaliplatin-related sinusoidal dilatation,^{31,40} supporting that sinusoidal endothelial cells play a crucial role at the early stage of sinusoidal dilatation. Given that VEGF is targeted by miR-150,⁴¹ sinusoidal dilatation-associated VEGF upregulation is consistent with the downregulation of miR-150 that we observed in the liver of patients with sinusoidal dilatation, and suggests that low miR-150 levels may contribute to the observed increase in VEGF. Similar to miR-150, miR-21 overexpression has been shown to suppress endothelial cell proliferation and migration of endothelial cells by inhibiting RhoB.⁴² In view of the important role that sinusoidal endothelial cells are playing in the development of sinusoidal dilatation, and the tight relationship between endothelial cells and miR-21/miR-150, our findings provide novel insight into the mechanisms of oxaliplatin-induced sinusoidal dilatation. Furthermore, given that microRNAs could be potential therapeutic options⁴³ to treat disease and treatment for sinusoidal dilatation is lacking, it is worth investigating that whether the occurrence of sinusoidal dilatation could be reduced by providing miR-21 and miR-150 as gene therapy.

The relationship between sinusoidal dilatation-associated microRNAs and survival is interesting. Although numerous studies reveal that miR-21 and miR-150 in tumour tissue play important roles in tumour proliferation and migration,^{33,44,41} which affects long-term survival,^{34,45} the impact of microRNAs in non-tumour liver parenchymal on the behavior of tumour is unknown. Given the suppressive action of miR-21 and miR-150 on endothelial cells, we could speculate that tumour angiogenesis occurring at the borderline area of normal liver and tumour may be inhibited, thereby contributing to increased long-term survival. However, in view of the cohort size, interpretation of findings concerning sinusoidal dilatation-related microRNAs in non-tumour liver and survival should be prudent, and further validation in a large cohort is encouraged and warranted. Because the downregulation of miR-21 and miR-150 in the liver of patients with sinusoidal dilatation was not found in plasma, these microRNAs are unlikely to be useful biomarkers of sinusoidal dilatation.

Chapter 7 describes the relationship between hepatic complement activation and the presence of CALI in patients with CRLM. 50 consecutive patients who underwent partial

hepatectomy for CRLM were included. Sinusoidal dilatation and nodular regeneration were assessed using formalin-fixed non-tumour liver samples. Several analyses were performed using frozen samples collected before manipulating the intestines or liver, which has been shown to limit inflammation due to sample collection.⁴⁶ C3c, C5a, and terminal complement complex (TCC) concentrations in liver tissue lysates were determined by ELISA to investigate complement activation; neutrophil infiltration was assessed by myeloperoxidase staining and subsequently quantified as a measure of lobular inflammation; hepatic triglyceride concentration was analysed to quantify steatosis. We found that hepatic C3c and C5a were both detectable, with C3c concentrations strongly correlating to C5a concentrations, consistent with persistent complement activation. Hepatic C5 activation was specifically increased in patients with nodular transformation and tended to be increased in patients with sinusoidal dilatation. Hepatic inflammation as reflected by neutrophil infiltration was markedly higher in patients with hepatic C3 and C5 activation. Steatosis was neither associated with activation of C3 nor with activation of C5. We concluded that complement activation occurs in the non-tumour liver of patients with CRLM, and that it was associated with the presence of hepatic nodular transformation and lobular inflammation.

Implications of Chapter 7

The thought of the complement system being involved in the development of steatosis and steatohepatitis was due to the fact that acylation stimulating protein, which is generated from C3a, stimulates triglyceride synthesis.⁴⁷ However, our data demonstrated that complement activation was only related to increased lobular inflammation but not hepatic triglyceride concentration, implying that complement activation only plays a role in hepatitis and the development of steatosis is probably not driven by mechanisms related to acylation stimulating protein. Our findings were supported by previous studies which show increased acylation stimulating protein⁴⁸ and activated complement system^{49,50} in steatohepatitis but not steatosis.

Although the cross-sectional design of the study precludes drawing conclusions on a causal link between complement activation and CALI, our data do provide evidence that various aspects of CALI are associated with complement activation. In view of the tight relationship between CALI and complement activation, and that diseases driven by complement activation could be treated with complement inhibitors,⁴⁹⁻⁵¹ it would be of interest to study the impact of complement inhibition on the prevention of CALI using existing drugs.

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Valorisation

Valorisation

To transfer academic research findings for the benefit of the society is one of the main tasks of Dutch universities by law.¹ Therefore, it is obligated to translate academic wisdom to societal benefit in ways that go beyond numerous academic findings. This is especially the case when research has been sponsored by foundations, non-profit organizations, taxpayers, or charities. This chapter is intended to describe how the research results presented in this thesis could be used in clinical practice and subsequently how a broadest community could benefit.

Relevance

Scientific relevance

Annually, approximately half of globally 1.3 million patients with colorectal cancer develop colorectal liver metastases (CRLM),²⁻⁴ of whom over 75% are not eligible for curative hepatic resection.^{4,5} Neoadjuvant chemotherapy enhances resectability by approximately 15% in this group,^{6,7} but has been associated with regimen-specific parenchymal liver injury, collectively referred to as chemotherapy-associated liver injury (CALI, i.e. sinusoidal dilatation, nodular regeneration, steatosis, and steatohepatitis).⁸⁻¹¹ Once occurred, CALI can persist for at least nine months even after cessation of chemotherapy.¹² The potential downstream side-effects of CALI on clinical outcome (i.e. postoperative morbidity, mortality, and long-term survival) after partial hepatectomy are an active area of research surrounded with controversy, and the molecular mechanisms underlying its development are still mostly unknown. Therefore, the central theme of the studies conducted in the present thesis was to unravel these questions.

Economic relevance

The current thesis has revealed that CALI is a risk factor for increased postoperative morbidity after partial hepatectomy for colorectal liver metastases. The presence of increased morbidity results in a prolonged length of hospital stay and may therefore eventually increase hospital cost. Moreover, in many cases, after discharge from the hospital, patients with postoperative morbidity remain to have the need of being followed by the first referral doctors (e.g. general practitioner) for a certain period. Therefore, it is reasonable to speculate that the increased cost as a consequence of extra health-care may eventually burden the government finance. Based on the findings from the current thesis, we were able to advise clinicians to adapt surgical management once CALI is diagnosed preoperatively or observed during liver surgery (e.g., 'blue' or

‘yellow’ liver). Therefore, the side-effects of CALI on postoperative morbidity and, as a consequence, the effects of CALI on the economic burden could be limited.

Societal relevance

Increased postoperative morbidity due to the presence of CALI has important potential negative influences on society. This is related to increased financial cost, but also to patients spending increased time on health-care related services, and, as a consequence, taking more sick leaves and spending less time on work. This negative influence may even radiate from the employer to the entire work-related network. Moreover, this thesis demonstrated that the presence of sinusoidal dilatation diminished tumour response to neoadjuvant chemotherapy. Of note, complete tumour response would allow patients to receive future curative surgical treatment and result in an increased tumour recurrence-free survival and overall survival. However, the diminished tumour response due to the occurrence of sinusoidal dilatation may alter the treatment strategy from curative to palliative. The latter, unfortunately, is related to a shorter lifespan. Additionally, being sick negatively affects quality of life, and the side effects of CALI are usually persistent for months.¹² Importantly, addition of bevacizumab to oxaliplatin-based chemotherapeutic regimens has been confirmed to be a beneficial approach to prevent sinusoidal dilatation, thereafter minimizing its negative effect on tumour response. Moreover, the development of oxaliplatin-induced sinusoidal dilatation, which we suggested to be related to the low expression of hepatic miR-21 and miR-150, may be prevented by treatment targeting these microRNAs in the future. With these evidence-based advices, patients would benefit from personalized treatment by prevented sinusoidal dilatation, increased tumour response, increased lifetime without tumour recurrence, and even increased lifespan. As a consequence, the negative influence of CALI on the society could be diminished.

Target groups

CRLM treatment involves multidisciplinary clinicians (e.g. gastroenterologists, surgeons, oncologists, and radiologists). The entire studies performed in this thesis focused on the population of patients with this disease. Therefore, in addition to the academic community, the research results benefit the group of patients with primary colorectal cancer and those with or without liver metastases. Additionally, our findings may also benefit paediatric patients who undergo hematopoietic stem cell transplantation and develop busulfan-induced hepatic sinusoidal injury^{13,14}; and those with breast cancer who develop tamoxifen-induced nonalcoholic steatohepatitis.¹⁵

Innovation and Implementation

The studies conducted in this thesis are novel and elaborated important clinical concerns. For instance, a first meta-analysis which aimed to study the downstream effect of CALI on postoperative morbidity was described in Chapter 2. Based on the findings from the previous chapter, Chapter 3 presents a first individual participant data analysis conducted on this topic. This new approach enables consistent statistical modelling and unified definitions for surgical outcomes when handling data from multiple international cohorts. Moreover, the relationship between CALI and postoperative infectious complications as shown in Chapter 4 was previously unknown in literature. Additionally, the links between microRNA profiles, complement activation, and the presence of CALI depicted in Chapter 6 and Chapter 7 provided novel insight into the mechanisms underlying its pathological features.

Because addition of bevacizumab to oxaliplatin-based regimens prevents the occurrence of sinusoidal dilatation, which has been demonstrated to be a risk factor for increased postoperative morbidity and diminished tumour response to neoadjuvant chemotherapy, it is advised to add bevacizumab to oxaliplatin-based treatment. Once CALI is diagnosed, treatment for patients with CRLM should be discussed in multidisciplinary meetings and surgical management is advised to be adapted (e.g. apply radiofrequency ablation and two-stage hepatectomy) to prevent the occurrence of morbidity. The role of miR-21 and miR-150 in oxaliplatin-induced sinusoidal dilatation is interesting, however, in view of the small cohort size, further validation in a large cohort is encouraged and warranted.

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Curriculum vitae

Curriculum vitae

J Zhao was born on the June 18th 1986 in Henan, China. After graduating from the first senior middle school in Queshan (June 2006), he was admitted by Zhengzhou University to study Clinical Medicine (seven-year-joint programme, September 2006).

From September 2006, he started his studies at the School of Basic Medicine at Zhengzhou University for two and half years. He was transferred to the department of Clinical Medicine for clinical curriculums in 2009. From 2010, he started his internship at the first affiliated hospital of Zhengzhou University. From 2011, he started to do his Master at the department of Hepatobiliary and Pancreatic Surgery under the supervision of Prof. Yongfu Zhao. In 2012, he was qualified as a medical practitioner (file no. 01241110412821198606181513). During the period of doing his Master, his interest in scientific research of the liver was sparked. In 2012, he awarded a national grant from the China Scholarship Council (file no. 201207040056). This grant sponsored his four-year PhD at the department of Surgery of Maastricht University under the supervision of Prof. Steven Olde Damink, Prof. Cornelis Dejong, and Sander Rensen, which resulted in this thesis. In November 2016, with the approval from the supervision team and the China Scholarship Council, he continued his research line at the department of Surgery of Maastricht University, while simultaneously facilitating international collaborations and training as a tutor of problem-based learning. In January 2017, he received an offer from Tongji hospital in Wuhan. With this offer, he will start his training by following the national standardized training protocol in October 2017 and be specialized at Biliary and Pancreatic Surgery in Tongji hospital afterwards.

List of publications

List of publications

Full papers

Zhao J*, van Mierlo KMC*, Gómez-Ramírez J, Kim H, Pilgrim CHC, Pessaux P, Rensen SS, van der Stok EP, Schaap FG, Soubrane O, Takamoto T, Viganò L, Winkens B, Dejong CHC, Olde Damink SWM; Chemotherapy-associated Liver Injury (CALI) consortium. Systematic review of the influence of chemotherapy-associated liver injury on outcome after partial hepatectomy for colorectal liver metastases. *British Journal of Surgery* 2017; 104(8):990-1002 (*contribute equally)

van Mierlo KMC*, **Zhao J***, Kleijnen J, Rensen SS, Schaap FG, Dejong CHC, Olde Damink SWM. The influence of chemotherapy-associated sinusoidal dilatation on short-term outcome after partial hepatectomy for colorectal liver metastases: a systematic review with meta-analysis. *Surgical Oncology* 2016; 25(3):298-307 (*contribute equally)

Zhao J, Zhao YF. Review of the mechanism underlying hepatic sinusoidal obstruction syndrome. *Henan Journal of Surgery* 2013; 19(4):81-82

Zhao J, Sawo P, Rouflart MMJ, Winstanley A, Vreuls CPH, Rensen SS, van Mierlo KMC, Lodewick TM, van Woerden V, van Tiel FH, van Dam RM, Dejong CHC, Olde Damink SWM. Impact of chemotherapy-associated liver injury on postoperative infectious complications after partial hepatectomy for patients with colorectal liver metastases. (submitted)

Zhao J, Sawo P, Rouflart MMJ, Winstanley A, Vreuls CPH, Rensen SS, van Mierlo KMC, Lodewick TM, van Woerden V, van Tiel FH, van Dam RM, Dejong CHC, Olde Damink SWM. Impact of chemotherapy-associated liver injury on tumour regression grade and survival after partial hepatectomy for patients with colorectal liver metastases. (submitted)

Zhao J, Rensen SS, Vreuls CPH, van den Broek MA, Driessen A, van Herwijnen M, Jetten MJA, Jennen DGJ, Dejong CHC, Olde Damink SWM. miR-21 and miR-150 downregulation is associated with oxaliplatin-related sinusoidal dilatation and impaired survival. (submitted)

Zhao J, Rensen SS, Vreuls CPH, Driessen A, van den Broek MA, Dejong CHC, Olde Damink SWM. Hepatic complement activation is associated with liver injury in patients with colorectal liver metastases. (submitted)

Conference abstracts

Zhao J, van Mierlo KMC, Rensen SS, Schaap FG, Dejong CHC, Olde Damink SWM, on behalf of Chemotherapy-associated Liver Injury (CALI) consortium. The influence of chemotherapy-associated liver injury on outcome after partial hepatectomy for colorectal liver metastases: a systematic review and individual patient data analysis. *Journal of Hepatology* 2016. Volume 64, issue 2, supplement, page S235. DOI: [http://dx.doi.org/10.1016/S0168-8278\(16\)00227-0](http://dx.doi.org/10.1016/S0168-8278(16)00227-0)

Zhao J, Rensen SS, Vreuls CPH, van den Broek MA, Driessen A, van Herwijnen M, Jetten MJA, Jennen DGJ, Dejong CHC, Olde Damink SWM. miR-21 and miR-150 downregulation is associated with oxaliplatin-related sinusoidal obstruction syndrome and impaired survival. *Journal of Hepatology* 2016. Volume 64, issue 2, supplement, page S567. DOI: [http://dx.doi.org/10.1016/S0168-8278\(16\)01027-8](http://dx.doi.org/10.1016/S0168-8278(16)01027-8)

Zhao J, van Mierlo KMC, Rensen SS, Schaap FG, Dejong CHC, Olde Damink SWM, on behalf of Chemotherapy-associated Liver Injury (CALI) consortium. The influence of chemotherapy-associated liver injury on outcome after partial hepatectomy for colorectal liver metastases: Results from a multicentre cohort study. *HPB* 2016. Volume 18, supplement 1, page e58. DOI: <http://dx.doi.org/10.1016/j.hpb.2016.02.142>

Zhao J, Rensen SS, Vreuls CPH, van den Broek MA, Jetten MJA, Coonen M, Jennen DGJ, van Herwijnen M, Dejong CHC, Olde Damink SWM. miR-21 and miR-150 downregulation correlates with oxaliplatin-related sinusoidal obstruction syndrome and impaired survival. *HPB* 2016. Volume 18, supplement 1, page e135. DOI: <http://dx.doi.org/10.1016/j.hpb.2016.02.318>

Zhao J, Sawo P, Rouflart MMJ, Rensen SS, van Mierlo KMC, Lodewick TM, van Woerden V, van Tiel FH, van Dam RM, Dejong CHC, Olde Damink SWM. Postoperative infectious complications decrease long-term survival after partial hepatic resection for colorectal liver metastases. *HPB* 2016. Volume 18, supplement 1, page e228. DOI: <http://dx.doi.org/10.1016/j.hpb.2016.02.569>

Scientific activities

Conference oral presentations

2017 (2) ALPS Liver and Pancreatic Surgery Meeting 2017 (Madonna di Campiglio, Italy)
Impact of chemotherapy-associated liver injury on tumour regression grade and survival in patients with colorectal liver metastases

2016 (4) 12th World Congress of the International Hepato-Pancreato Biliary Association (IHPBA, Sao Paulo, Brazil) The influence of chemotherapy-associated liver injury on outcome after partial hepatectomy for colorectal liver metastases: a systematic review and individual patient data analysis

2015 (11) 12th International Medical Postgraduate Conference (Prague, Czech Republic)
miR-21 and miR-150 downregulation is associated with oxaliplatin-related sinusoidal obstruction syndrome and impaired survival

2015 (10) Dutch Society for Gastroenterology (NVGE, Veldhoven, the Netherlands)
Postoperative infectious complications decrease long-term survival after partial hepatic resection for colorectal liver metastases

2015 (10) Dutch Society for Gastroenterology (NVGE, Veldhoven, the Netherlands) The influence of chemotherapy-associated liver injury on outcome after partial hepatectomy for colorectal liver metastases: systematic review and pooled analysis of individual patient data

2014 (10) Dutch Society for Gastroenterology (NVGE, Veldhoven, the Netherlands)
Oxaliplatin related sinusoidal obstruction syndrome is correlated with down-regulation of miR-21 and miR-150

Conference posters

2016 (4) 12th World Congress of the International Hepato-Pancreato Biliary Association (IHPBA, Sao Paulo, Brazil) miR-21 and miR-150 downregulation is associated with oxaliplatin-related sinusoidal obstruction syndrome and impaired survival

2016 (4) 12th World Congress of the International Hepato-Pancreato Biliary Association (IHPBA, Sao Paulo, Brazil) Postoperative infectious complications decrease long-term survival after partial hepatic resection for colorectal liver metastases

2016 (4) The International Liver Congress 2016 (EASL, Barcelona, Spain) miR-21 and miR-150 downregulation is associated with oxaliplatin-related sinusoidal obstruction syndrome and impaired survival

2016 (4) The International Liver Congress 2016 (EASL, Barcelona, Spain) The influence of chemotherapy-associated liver injury on outcome after partial hepatectomy for colorectal liver metastases: a systematic review and individual patient data analysis

2016 (3) Dutch Society for Gastroenterology (NVGE, Veldhoven, the Netherlands) Complement activation in patients with chemotherapy-associated steatosis and steatohepatitis