Maastricht University

Postoperative liver (dys)function

Citation for published version (APA):

van Mierlo, K. M. C. (2022). *Postoperative liver (dys)function: determinants and interventions*. [Doctoral Thesis, Maastricht University]. Maastricht University. https://doi.org/10.26481/dis.20220708km

Document status and date:
Published: 01/01/2022

DOI:
10.26481/dis.20220708km

Document Version:
Publisher's PDF, also known as Version of record

Please check the document version of this publication:

• A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
• The final author version and the galley proof are versions of the publication after peer review.
• The final published version features the final layout of the paper including the volume, issue and page numbers.

Link to publication

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the “Taverne” license above, please follow below link for the End User Agreement:

www.umlib.nl/taverne-license

Take down policy
If you believe that this document breaches copyright please contact us at:

repository@maastrichtuniversity.nl

providing details and we will investigate your claim.

Download date: 10 Apr. 2024

Postoperative liver (dys)function: determinants and interventions

Kim M.C. van Mierlo

Cover design: Kim van Mierlo, onder leiding van Corné Akkers (www.corneakkers.com)
Printed by: Optima Grafische Communicatie (www.ogc.nl)
ISBN 978-94-6361-711-6

Instanties die financieel aan de totstandkoming van het proefschrift hebben bijgedragen:
Nederlandse Vereniging van Hepatologie
Universiteit Maastricht

Copyright © 2022 Kim van Mierlo
All right reserved. No part of this dissertation may be reproduced, distributed, of transmitted in any form or by any means, including photocopying, recording, or other electronic or mechanical methods, without the prior written permission of the author or publisher, except in case of brief quotations embodied in critical reviews and certain other non-commercial uses permitted by copyright law

Postoperative liver (dys)function:
determinants and interventions

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Universiteit Maastricht,
op gezag van de Rector Magnificus, prof. dr. Pamela Habibović,
volgens het besluit van het College van Decanen,
in het openbaar te verdedigen
op vrijdag 8 juli 2022 om 13.00 uur

door

Kim Maria Cornelia van Mierlo

Promotores
Prof. dr. S.W.M. Olde Damink
Prof. dr. C.H.C. Dejong

Copromotor
Dr. F.G. Schaap

Beoordelingscommissie
Prof. dr. I.C.C. van der Horst (voorzitter)
Dr. M.T. de Boer (Universitair Medisch Centrum Groningen)
Prof. dr. W.F.F.A. Buhre
Dr. J.I. Erdmann (Amsterdam Universitair Medisch Centrum)
Prof. dr. R. Shiri-Sverdlov

Table of contents

Chapter 1

General introduction, aims and outline of this thesis

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] In addition, the incidence of primary liver cancer (hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA)) is also increasing with over 700 new cases per annum in The Netherlands alone.[3] Partial liver resection for hepatobiliary tumours is often the only curative treatment option. The regenerative capacity of the liver permits the surgical removal of a substantial part of the liver mass. Although liver resection provides the best prospect of cure, only 10-30% of patients with CRLM are eligible for hepatic surgery.[4] Inoperability is mainly caused by patient (co)morbidity and future remnant liver volume (RLV) that results from the combination of number and size of hepatic tumours. To reduce tumour load and enhance accessibility to curative surgery, neoadjuvant chemotherapy can be administered.

Liver regeneration after partial hepatectomy

Liver regeneration following partial hepatectomy is a tightly orchestrated process involving the spatiotemporal interplay between parenchymal and non-parenchymal cells and is driven by multiple signals.[5] Immediately after partial liver resection, the total hepatic inflow passes through the vascular bed of the smaller remnant liver. Resultant shear stress, a relative increase in supply of signalling molecules from the (portal) circulation, and growth factors released after remodelling of the extracellular matrix, provide the triggers for initiation of liver regeneration. Interleukin 6 and tumour necrosis factor alpha released by activated Kupffer cells are important for cell cycle re-entry of normally quiescent hepatocytes, with further cell cycle progression driven by mitogens such as hepatocyte growth factor. Proliferation of the various non-parenchymal cell types enables re-establishment of the hepatic architecture. Through poorly understood molecular events, liver regeneration terminates when the original liver mass and functional capacity have been restored.[6]

Impaired liver regeneration

During liver regeneration, a minimum amount of remnant liver is required to maintain vital liver functions and support regrowth. In a seminal study, almost half of the patients with a RLV smaller than 26.6% of the pre-resection value, developed severe hepatic dysfunction compared with 1.2% of patients with a larger RLV.[2] Consequently, a RLV of 25–30% is currently used as lower limit in patients with normal liver function, whereas a minimum RLV of about 40% is required in patients with impaired liver function.[4] An imbalance between liver volume and quality, with lack of functional recovery after (extended) resection, may lead to postresectional liver failure (PLF). PLF is characterized by an impaired synthetic, secretory, and detoxifying function of the liver, and accounts for

most of the mortality after extensive hepatectomy.[1] Five main factors have been recognized in the aetiology of PLF. Next to hepatic haemodynamic imbalance, an impaired liver innate immune response, the gut microbiome-gut-liver axis, disturbed bile salt homeostasis and background liver dysfunction play an important role in the development of PLF. For this thesis, we will only discuss the latter two items.

Unmet hepatic metabolic demand: disturbed bile salt homeostasis

Impaired activity of the canalicular pump(s) involved in bilirubin secretion results in intrahepatic accumulation and systemic release of conjugated bilirubin.[7] While bilirubin is generally not regarded as detrimental to the liver, a more generalized dysfunction of canalicular transporters may result in hepatic accumulation of bile salts. Circulating levels of bile salts rise as early as one minute after partial hepatectomy in rats,[8] and this is shortly followed by transient accumulation of bile salts in the liver.[9] An important stimulatory role for bile salts and their membrane-bound and nuclear receptors in liver regeneration is emerging.[10] Being biological detergents, excessive intracellular accumulation of bile salts, however, causes damage to internal membranes (particularly in mitochondria) of the hepatocyte and results in (controlled) cell death and immunological sequela.[11] In mice with deranged bile salt homeostasis, an otherwise well-tolerated 70% partial hepatectomy procedure results in massive hepatocyte necrosis and early mortality.[12] Animal studies underscore that tight control of (hepatic) bile salt homeostasis is a prerequisite for unimpeded liver regeneration.[12,13]

Impaired background liver function

Neoadjuvant chemotherapy is widely used for downstaging of CRLM, enabling subsequent surgical tumour removal. Chemotherapy-associated liver injury (CALI) is often reported in patients with CRLM and appears to be regimen specific. Oxaliplatin is central in most currently used regimens and is considered the main causative agent for development of sinusoidal obstruction syndrome (SOS) and nodular regenerative hyperplasia, whereas irinotecan has been associated with the development of chemotherapy-associated steatohepatitis. SOS is seen in up to 80% of patients undergoing oxaliplatin-based chemotherapy,[14] and is characterized by injury of endothelial cells, parenchymal damage, and (fibrotic) venular lesions. Although exact mechanisms are unclear, a diminished preoperative functional reserve and longer hospital stay after major hepatectomy were reported in patients with sinusoidal dilatation.[15] The effect of CALI on development of PLF is uncertain.[16,17]

Preoperative assessment of liver volume and function

Both assessment of liver volume and quality is mandatory to predict postoperative functional reserve. Methods for measurement of RLV range from 2D volumetry on computed

tomography, to perioperative 3D modelling. Computational software allows the radiologist or surgeon to delineate the liver manually or automatically on all CT or MRI sections, thereby allowing calculation of liver volume.[18,19]

Liver function can be estimated by preoperative serum tests, breath tests and imaging. Hepatic secretory, synthetic, and detoxifying functions and liver damage are indicated by serum bilirubin, INR, ALT, AST, ammonia, and various metabolites. Metabolic liver function testing can be performed with the LiMAx test and the indocyanine green clearance rate (ICGR-15). Imaging techniques used in the clinic include ^{99m}Tc-labeled GSA liver scintigraphy, ^{99m}Tc-mebrofenin hepatobiliary scintigraphy with SPECT, and gadolinium-enhanced MRI using Gd-EOB-DTPA.

Postoperative detection of PLF

Several risk scores have been developed to detect liver failure postoperatively. Hyperbilirubinemia is included in all currently used definitions of PLF. The '50-50 criteria' predict a 59% risk on early postoperative mortality if systemic bilirubin rises above 50 µmol/L and prothrombin time decreases below 50% on postoperative day 5.[20] The 'peak bilirubin criterion' defines PLF as a bilirubin level above 120 µmol/L within 90 days after major hepatectomy, and has a positive predictive value of 33% for liver-related death in non-cirrhotic patients.[21] The definition of PLF developed by the International Study Group of Liver Surgery encompasses bilirubin elevation (according to local criteria) on or after postoperative day 5, and grades PLF based on international normalized ratio (INR) derangement.[22] Postoperative mortality in PLF grade A (INR <1.5), B (INR ≥1.5 and <2.0) and C (INR ≥2.0) was 0%, 12%, and 54%, respectively.

Once PLF is detected, only symptomatic treatment can be provided. Preoperative prevention of PLF is therefore of uttermost importance.

Prevention of PLF - Surgical targeting

Hypertrophy-inducing procedures and surgical adaptations should be performed if the RLV is expected to be <25% in patients without liver disease and <35-40% in patients with impaired liver function.[23] In general, portal vein embolization enlarges the RLV with approximately 30-40% and improves eligibility for hepatectomy by 20%.[24] A recently described method is combined preoperative portal and hepatic vein embolization (bi-embolization), which induces more hypertrophy (51%) than portal vein embolization before major liver resection with no more morbidity.[25] The two-staged hepatectomy is an excellent method to increase RLV and consequently achieve curation in patients with bilobar tumours, who are not deemed resectable in a single attempt. PVL concurrent with two-staged hepatectomy resulted in an RLV gain of about 40% after eight weeks.

The recently developed associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) procedure is based on the same principle as two-staged hepatectomy, albeit that during the first stage in ALPPS the liver is split *in situ* combined with portal vein ligation, and the second stage consists of removal of the embolized ligated lobe 7-14 days later.[26] An astonishing average hypertrophy rate of 80% can be achieved with this procedure, creating a curative opportunity for initially non-resectable patients who have insufficient (predicted) hypertrophy on portal vein embolization.[27] Although ALPPS is currently performed in all liver backgrounds, histological changes comprising fibrosis, steatosis, and chemotherapy-related alterations resulted in lower hypertrophy rates and considerate morbidity and mortality.[27] It should therefore be used with caution. In cholestatic liver, the procedure is advised against.

Targeting molecular receptors

In recent years, bile salts have emerged as important signalling molecules in liver regeneration (LR). The nuclear bile salt receptor Farnesoid X Receptor (FXR) may be an attractive therapeutic candidate, through effects on hepatic haemodynamics, bile salt and lipid homeostasis, hepatic inflammation, and hepatocellular proliferation. Being the key regulator of hepatic bile salt homeostasis, genetic disruption of *Fxr* in mice resulted in mortality and delayed liver regeneration after partial hepatectomy.[12] Conversely, activation of Fxr by its endogenous ligands (i.e. bile salts) or synthetic agonists enhanced liver regeneration in hepatectomized mice.[12] Furthermore, the FXR-regulated enterokine FGF19 improved bile salt homeostasis and reduced mortality in an extended hepatectomy mouse model of acute liver failure.[12] FXR agonists (as well as an engineered FGF19 variant) undergo current clinical evaluation, and already showed efficacy in halting fibrotic progression in NASH patients.[28]

AIMS OF THIS THESIS

The central aim of this thesis was to study determinants of and interventions on postresectional liver (dys)function after partial hepatectomy for liver cancer. This was subdivided into three specific research areas; 1) to study the impact of chemotherapy-associated liver injury on morbidity and mortality after partial hepatectomy for liver cancer, 2) to investigate current and future (functional) endpoints to define and detect postresectional liver failure, and 3) to examine the role of bile salts and nuclear FXR agonism in (the prevention of) liver failure and acceleration of postresectional liver regeneration.

OUTLINE OF THIS THESIS

Postresectional liver failure is a feared complication in patients after partial hepatectomy. In **Chapter 2** we review the pathophysiology, prediction, and prevention of PLF. The majority of patients undergoing partial hepatectomy for CRLM receive preoperative chemotherapy to reduce tumour load. Chemotherapy-induced liver injury is a histopathological entity that occurs in up to three quarters of patients who receive chemotherapy for CRLM. In **Chapter 3** we assess current literature on the influence of chemotherapy-associated sinusoidal dilatation on short-term complications in patients who underwent partial hepatectomy for CRLM. We noted that the current body of literature falls short on comparable inclusion criteria, histological assessment, and study endpoints. To overcome this shortcoming, we developed a collaborative database with individual participant data consisting of multiple, international data cohorts. In **Chapter 4** we elaborate further on the influence of chemotherapy-associated liver injury (sinusoidal dilatation, steatosis, and steatohepatitis) on short-term outcome in patients undergoing partial hepatectomy for CRLM in an individual participant data analysis.

Although the safety of liver surgery has improved tremendously, hepatic surgery continues to face challenging complications. The conduct of randomized controlled trials in liver surgery using dichotomous outcomes requires a large sample size. In **part II**, we focus on surrogates to detect postresectional liver dysfunction. The use of surrogate endpoints (SEPs) reduces sample size but SEPs should be validated before use. In **Chapter 5** we investigate currently used SEPs in liver surgery trials. One of the main definitions of PLF is the occurrence of a bilirubin level >120 μmol/L within 90 days after partial hepatectomy. In **Chapter 6** we validate this criterion as a risk indicator for postresectional morbidity and liver-related mortality. Lastly, we focused on preoperative determination of liver function to prevent PLF. In **chapter 7** we challenged the liver of patients with CRLM with acetaminophen to test potential of ophthalmic acid level as a prediction read-out to evaluate preoperative liver function.

Despite pre- and postoperative measures to prevent PLF, it is still the most common cause of mortality after partial hepatectomy. Once fulminantly present, only supportive life care can be given. Animal models mimicking the human situation would allow us to evaluate pharmacological options to prevent or overcome PLF and are described in **part III** of this thesis. In this part, we focused on the role of bile salts in liver regeneration and postresectional liver dysfunction. Although bile salts seem required for proper postresectional liver regeneration, a tight control of intracellular levels seems indispensable as shown by increased morbidity and mortality in rodent models with an overload of bile salts. First, we developed a rodent model with a deranged bile salt homeostasis to mimic

PLF (**Chapter 8**). FGF19, which is induced upon bile salt stimulation of FXR, has been recognized as a potent mitogen and regulator of bile salt homeostasis. In **Chapter 9** we hypothesize that improvement of bile salt homeostasis via FXR-agonism accelerates liver regeneration after partial hepatectomy in rodent models. **Chapter 10** summarizes our findings and encompasses future perspectives and implications.

REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; **136**(5): E359-86.
2. Kanas GP, Taylor A, Primrose JN, et al. Survival after liver resection in metastatic colorectal cancer: review and meta-analysis of prognostic factors. *Clin Epidemiol* 2012; **4**: 283-301.
3. IKNL – Cijfers over kanker. Incidentie | Lever en intrahepatische galwegen L, 2018.
4. van den Broek MA, Olde Damink SW, Dejong CH, et al. Liver failure after partial hepatic resection: definition, pathophysiology, risk factors and treatment. *Liver international* 2008; **28**(6): 767-80.
5. Fausto N, Campbell JS, Riehle KJ. Liver regeneration. *J Hepatol* 2012; **57**(3): 692-4.
6. Michalopoulos GK. Principles of liver regeneration and growth homeostasis. *Compr Physiol* 2013; **3**(1): 485-513.
7. Erlinger S, Arias IM, Dhumeaux D. Inherited disorders of bilirubin transport and conjugation: new insights into molecular mechanisms and consequences. *Gastroenterology* 2014; **146**(7): 1625-38.
8. Doignon I, Julien B, Serriere-Lanneau V, et al. Immediate neuroendocrine signaling after partial hepatectomy through acute portal hyperpressure and cholestasis. *J Hepatol* 2011; **54**(3): 481-8.
9. Pean N, Doignon I, Garcin I, et al. The receptor TGR5 protects the liver from bile acid overload during liver regeneration in mice. *Hepatology* 2013; **58**(4): 1451-60.
10. Huang W, Ma K, Zhang J, et al. Nuclear receptor-dependent bile acid signaling is required for normal liver regeneration. *Science* 2006; **312**(5771): 233-6.
11. Perez MJ, Briz O. Bile-acid-induced cell injury and protection. *World J Gastroenterol* 2009; **15**(14): 1677-89.
12. Uriarte I, Fernandez-Barrena MG, Monte MJ, et al. Identification of fibroblast growth factor 15 as a novel mediator of liver regeneration and its application in the prevention of post-resection liver failure in mice. *Gut* 2013; **62**(6): 899-910.
13. Fan M, Wang X, Xu G, Yan Q, Huang W. Bile acid signaling and liver regeneration. *Biochim Biophys Acta* 2015; **1849**(2): 196-200.
14. Rubbia-Brandt L, Audard V, Sartoretti P, et al. Severe hepatic sinusoidal obstruction associated with oxaliplatin-based chemotherapy in patients with metastatic colorectal cancer. *Ann Oncol* 2004; **15**(3): 460-6.
15. Nakano H, Oussoultzoglou E, Rosso E, et al. Sinusoidal injury increases morbidity after major hepatectomy in patients with colorectal liver metastases receiving preoperative chemotherapy. *Ann Surg* 2008; **247**(1): 118-24.
16. Soubrane O, Brouquet A, Zalinski S, et al. Predicting high grade lesions of sinusoidal obstruction syndrome related to oxaliplatin-based chemotherapy for colorectal liver metastases: correlation with post-hepatectomy outcome. *Ann Surg* 2010; **251**(3): 454-60.
17. Vigano L, Ravarino N, Ferrero A, Motta M, Torchio B, Capussotti L. Prospective evaluation of accuracy of liver biopsy findings in the identification of chemotherapy-associated liver injuries. *Arch Surg* 2012; **147**(12): 1085-91.
18. van der Vorst JR, van Dam RM, van Stiphout RS, et al. Virtual liver resection and volumetric analysis of the future liver remnant using open source image processing software. *World J Surg* 2010; **34**(10): 2426-33.
19. Dello SA, van Dam RM, Slangen JJ, et al. Liver volumetry plug and play: do it yourself with ImageJ. *World J Surg* 2007; **31**(11): 2215-21.
20. Balzan S, Belghiti J, Farges O, et al. The "50-50 criteria" on postoperative day 5: an accurate predictor of liver failure and death after hepatectomy. *Ann Surg* 2005; **242**(6): 824-8

21. Mullen JT, Ribero D, Reddy SK, et al. Hepatic insufficiency and mortality in 1,059 noncirrhotic patients undergoing major hepatectomy. *J Am Coll Surg* 2007; **204**(5): 854-62;
22. Rahbari NN, Garden OJ, Padbury R, et al. Posthepatectomy liver failure: a definition and grading by the International Study Group of Liver Surgery (ISGLS). *Surgery* 2011; **149**(5): 713-24.
23. Vauthey JN, Abbott DE. Commentary on "Feasibility study of two-stage hepatectomy for bilobar liver metastases". *Am J Surg* 2012; **203**(6): 698-9.
24. van den Broek MA, Olde Damink SW, Dejong CH, et al. Liver failure after partial hepatic resection: definition, pathophysiology, risk factors and treatment. *Liver Int* 2008; **28**(6): 767-80.
25. Le Roy B, Gallon A, Cauchy F, et al. Combined biembolization induces higher hypertrophy than portal vein embolization before major liver resection. *HPB* 2019.
26. Schnitzbauer AA, Lang SA, Goessmann H, et al. Right portal vein ligation combined with in situ splitting induces rapid left lateral liver lobe hypertrophy enabling 2-staged extended right hepatic resection in small-for-size settings. *Ann Surg* 2012; **255**(3): 405-14.
27. Schadde E, Ardiles V, Robles-Campos R, et al. Early survival and safety of ALPPS: first report of the International ALPPS Registry. *Ann Surg* 2014; **260**(5): 829-36
28. Neuschwander-Tetri BA, Loomba R, Sanyal AJ, et al. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. *Lancet* 2015; **385**(9972): 956-65.

PART I

Preoperative determinants of postoperative liver function

Chapter 2

Liver resection for cancer: new developments in prediction, prevention and management of postresectional liver failure

Kim M.C. van Mierlo, Frank G. Schaap, Cornelis H.C. Dejong, Steven W.M. Olde Damink

Journal of Hepatology. 2016 Dec;65(6):1217-1231

SUMMARY

Hepatic failure is a feared complication that accounts for up to 75% of mortality after extensive liver resection. Despite improved perioperative care, the increasing complexity and extensiveness of surgical interventions, in combination with an expanding number of resections in patients with compromised liver function, still results in an incidence of postresectional liver failure (PLF) of 1–9%. Preventive measures aim to enhance future remnant liver size and function. Numerous non-invasive techniques to assess liver function and predict remnant liver volume are being developed, along with introduction of novel surgical strategies that augment growth of the future remnant liver. Detection of PLF is often too late and treatment is primarily symptomatic. Current therapeutic research focuses on ([bio] artificial) liver function support and regenerative medicine. In this review we discuss the current state and new developments in prediction, prevention, and management of PLF, in light of novel insights into the aetiology of this complex syndrome.

INTRODUCTION

Partial liver resection for hepatobiliary tumours is relatively safe and often the only curative treatment option. The unequalled capacity of the liver to regenerate and restore its functionalities permits the surgical removal of a substantial part of the liver mass. However, postresectional liver failure (PLF) occurs in up to 9% of patients and remains the main cause of postoperative mortality.[1,2] PLF has a subacute course, and an inadequate functional reserve of the remnant liver is central in its aetiology. Insufficient hepatic secretory capacity is reflected by hyperbilirubinemia, whereas decreased synthetic and detoxifying functions can manifest as coagulopathy and hepatic encephalopathy.[1] Hyperbilirubinemia is included in all currently used definitions of PLF. The '50–50 criteria' predict a 59% risk on early postoperative mortality if systemic bilirubin rises above 50 µmol/L and prothrombin time decreases to 50% on postoperative day 5.[3] The 'peak bilirubin criterion' defines PLF as a bilirubin level above 120 µmol/L within 90 days after major hepatectomy, and has a positive predictive value of 33% for liver-related death in non-cirrhotic patients.[4] The definition of PLF developed by the International Study Group of Liver Surgery encompasses bilirubin elevation (according to local criteria) on or after postoperative day 5, and grades PLF based on international normalized ratio (INR) derangement.[5] Postoperative mortality in PLF grade A (INR <1.5), B (INR >1.5 and <2.0) and C (INR >2.0) was 0%, 12%, and 54%, respectively.[5] In order to provide a comprehensive overview of this syndrome, no specific definition was selected for this review.

LIVER REGENERATION AFTER PARTIAL LIVER RESECTION

Liver regeneration following partial hepatectomy is a tightly orchestrated process involving the spatiotemporal interplay between parenchymal and non-parenchymal cells and is driven by multiple signals (see for detailed reviews references 6 and 7). First, immediately after partial liver resection, the total hepatic inflow passes through the vascular bed of the smaller remnant liver. Resultant shear stress, a relative increase in supply of signalling molecules from the (portal) circulation, and growth factors released after remodelling of the extracellular matrix, provide the triggers for initiation of liver regeneration. Interleukin 6 and tumour necrosis factor alpha released by activated Kupffer cells are important for cell cycle re-entry of normally quiescent hepatocytes, with further cell cycle progression driven by mitogens such as hepatocyte growth factor. Proliferation of the various nonparenchymal cell types enables re-establishment of the hepatic architecture. Through poorly understood molecular events, liver regeneration terminates when the original liver mass and functional capacity have been restored.

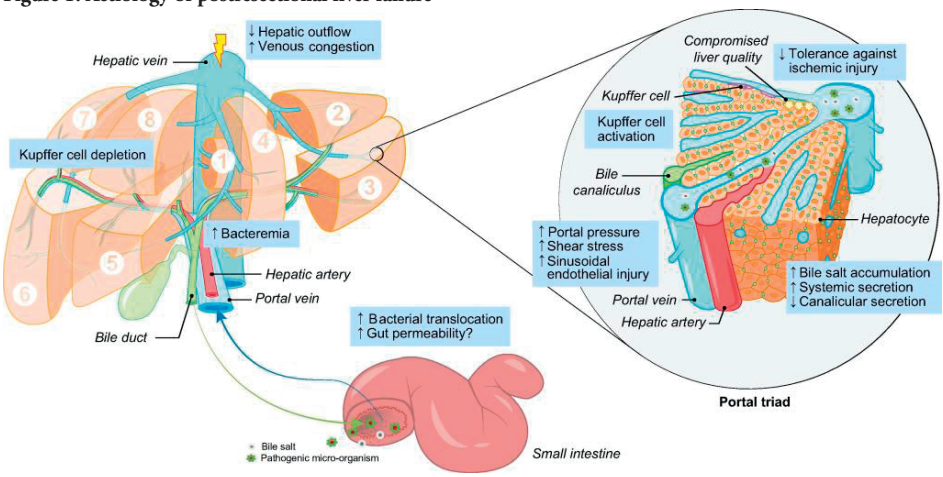
AETIOLOGY OF POSTRESECTIONAL LIVER FAILURE

During liver regeneration, a minimum amount of remnant liver is required to maintain vital liver functions and support regrowth. In a seminal study almost half of the patients with a remnant liver volume (RLV) smaller than 26.6% of the pre-resection value, developed severe hepatic dysfunction compared with 1.2% of patients with a larger RLV.[2] Consequently, a RLV of 25–30% is currently used as lower limit in patients with normal liver function, whereas a minimum RLV of about 40% is mandatory in patients with impaired liver function.[8] Five main factors have been recognized in the aetiology of PLF (Figure 1).

Hepatic haemodynamic imbalance

PLF shares features of the small-for-size syndrome that occurs in the setting of (partial) liver transplantation. Portal hyperperfusion of the remnant liver results in adaptive reduction of arterial blood flow through activation of the hepatic arterial buffer response (see reference 9 for a detailed review). While increased perfusion and resultant shear stress are instrumental in initiating the regenerative cascade, portal hyperperfusion and arterial hypoperfusion may have deleterious effects on postoperative recovery of liver function.[9] Increased portal flow and pressure after major hepatectomy increased the risk for PLF in non-cirrhotic patients.[10] In patients undergoing partial liver transplantation, post-reperfusion portal hypertension resulted in sinusoidal damage and reduced levels of nitric oxide, a signal molecule engaged in the initiation of liver regeneration.[11]

Figure 1. Aetiology of postresectional liver failure



The altered blood-to-liver volume ratio causes elevated portal pressure and resultant shear stress and sinusoidal endothelial injury. Although Kupffer cells are activated, activity in the liver remnant is inadequate to initiate and/or maintain the innate immune response that drives postresectional liver regeneration. Combined with increased enteric bacterial translocation, the infectious risk is increased. Impaired canalicular secretion of bile salts results in intrahepatic accumulation and subsequent hepatocellular injury. In case of venous reconstruction, impaired hepatic outflow can result in hepatic venous congestion. Lastly, livers with compromised function due to chronic liver diseases are more vulnerable to perioperative ischemic reperfusion injury, as reflected in impaired recovery of postoperative liver function

Unmet hepatic metabolic demand: disturbed bile salt homeostasis

Impaired activity of the canalicular pump(s) involved in bilirubin secretion results in intrahepatic accumulation and systemic release of conjugated bilirubin.[12] While bilirubin is generally not regarded as detrimental to the liver, a more generalized dysfunction of canalicular transporters may result in hepatic accumulation of bile salts. Circulating levels of bile salts rise as early as one minute after partial hepatectomy in rats,[13] and this is shortly followed by transient accumulation of bile salts in the liver.[14] An important stimulatory role for bile salts and their membrane-bound and nuclear receptors in liver regeneration is emerging.[15] Being biological detergents, excessive intracellular accumulation of bile salts, however, causes damage to internal membranes (particularly in mitochondria) of the hepatocyte and results in apoptosis.[16] In mice with deranged bile salt homeostasis, otherwise well-tolerated 70% partial hepatectomy results in massive hepatocyte necrosis and early mortality.[17] Animal studies underscore that tight control of (hepatic) bile salt homeostasis is a prerequisite for unimpeded liver regeneration.[17,18]

Impaired liver innate immune defence

Liver regeneration after partial hepatectomy involves activation of the livers' innate immune system.[19] Innate immune receptors of the Toll-like receptor family that recognize bacterial products, and downstream (adaptor) proteins that relay the signal intracellularly, are engaged in this activation step.[20] Liver-resident macrophages not only play an important

role in the regenerative response after liver resection by producing priming factors, they also clear portal endotoxins and eliminate translocated bacteria,[21] thus limiting exposure of hepatocytes to (pro-apoptotic) lipopolysaccharide (LPS) and preventing systemic infection.[22] Following resection, adequate numbers of Kupffer cells should remain to preserve these essential functions. The risk of infection increases with the extent of resection, and the majority of patients with hepatic dysfunction also develops infectious complications.[2] Cytokine release by activated Kupffer cells is hampered after major liver resection.[22] Likewise, impaired phagocytic activity of the reticuloendothelial system is observed after major resection,[23] and this likely contributes to increased infectious risk.[2]

Gut microbiome-gut-liver axis

An emerging concept is that the gut microbiota modulates the regenerative ability of the liver (reviewed in reference 24). This is likely to involve interactions between the gut microbiome and host metabolism, effects of the gut microbiota on bile salt physiology, as well as effects of bacterial endotoxins.[24,25] Bile salts exert direct antimicrobial activity and shape the composition of the gut flora. Conversely, certain microbial strains can convert the host's primary bile salts into secondary species, thus affecting the signalling properties of bile salts. This again can impact host metabolism, bile salt homeostasis, and liver regeneration.[26-28] As discussed above, activation of the innate immune response in the liver is important for liver regeneration after partial hepatectomy, and microbial products including LPS are implicated in Kupffer cell activation.[29] Failure of gut-derived endotoxins to reach the liver resulted in impaired DNA synthesis in replicating hepatocytes, likely through reduced production of priming factors.[30] On the other hand, excessive levels of endotoxin can impair liver regeneration and cause mortality after extended hepatectomy.

Impaired background liver function

Impaired liver quality plays a pivotal role in PLF and is frequently present in patients that undergo partial hepatectomy for the three most common indications: colorectal cancer liver metastasis (CRLM), hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA). Four types of liver pathology are related to these hepatobiliary tumours, viz. chemotherapy-associated liver injury, fatty degeneration, fibrotic progression, and cholestasis.

Chemotherapy-associated liver injury (CALI)

Neoadjuvant chemotherapy is widely used for downstaging of CRLM. Oxaliplatin is central in most currently used regimens and is considered the main causative agent for development of sinusoidal obstruction syndrome (SOS) and nodular regenerative hyperplasia (NRH), whereas irinotecan has been associated with the development of chemotherapy-associated steatohepatitis. SOS is seen in up to 80% of patients undergo-

ing oxaliplatin-based chemotherapy,[31] and is characterized by injury of endothelial cells, parenchymal damage, and (fibrotic) venular lesions. Sinusoidal dilatation (SD) is the most common manifestation of SOS in the grading system of Rubbia-Brandt et al.[31]

Although exact mechanisms are unclear, a diminished preoperative functional reserve and longer hospital stay after major hepatectomy were reported in patients with SD.[32] The effect of SD on development of PLF is uncertain. Studies indicate no effect, or an incidence of PLF in up to 21% of patients with moderate to severe SD after major hepatectomy (0–4.2% in patients with absent or mild SD).[33,34] Rodent models using monocrotalin or oxaliplatin to induce SOS revealed impairment of liver regeneration and induction of liver injury following partial hepatectomy.[35] This was accompanied by less pronounced induction of hepatic mitogens, reduced liver volume recovery, enhanced hepatocellular necrosis and higher serum alanine aminotransferase (ALT) and bilirubin levels.[35,36] Bevacizumab, a monoclonal antibody directed against vascular endothelial growth factor A, decreased the incidence of SD in patients that received oxaliplatin-based chemotherapy.[37] Downregulation of matrix metallopeptidase-9, a fibrotic remodelling factor involved in perisinusoidal extracellular matrix breakdown, may be accountable.[38]

SD is a histological diagnosis and can be detected by biopsy, however the false-negative classification is high due to the spatial heterogeneity of its manifestation.[34] Surrogate measures are biochemical assessment, functional tests, imaging and spleen size measurement. Increased aspartate aminotransferase (AST) and alkaline phosphatase (ALP) levels can point to SD but are non-specific. An indocyanine green retention rate after 15 min (ICG-R15) >10% and the preoperative AST-to-platelet-ratio index (APRI) are both independently associated with the presence of SD.[32,33] Patients with SD often have an increased spleen volume,[39] with an increase of 30% reported to be predictive of SD.[40] Another typical SD-related abnormality seen in imaging is reticular hypointensity that presumably reflects locally impaired Kupffer cell function.[41] Superparamagnetic iron oxide-enhanced MRI can detect moderate to severe SD,[41] but gadoxetic acid enhanced MRI seems superior with a specificity of 96–100% on hepatobiliary phase images.[42]

A second histological characteristic of SOS is NRH, which is observed in over 24% of patients after oxaliplatin-based chemotherapy.[38] NRH is characterized by diffuse transformation of liver parenchyma into regenerative nodules that compress the surrounding parenchyma, and is graded according to Wanless et al.[43] NRH probably arises due to changes in intrahepatic sinusoidal or portal blood flow.[43] The incidence of PLF is increased in patients with NRH, even rising to 25% after major hepatectomy in patients with moderate to severe NRH.[44] Furthermore, coexistence of NRH with moderate to severe SD has been noted.[44] Since SD, in contrast to NRH, was no indisputable risk

indicator for postoperative outcome, it was suggested that NRH is the true determinant of poor short-term outcome after liver resection. Although the mechanism is not elucidated, hepatic injury, portal hypertension and a lower platelet count may predispose to PLF.[45] A decreased platelet count combined with elevated ALP, gamma-glutamyl transferase (GGT), and total bilirubin levels can be found in NRH.[46] APRI can predict NRH.[44] Percutaneous or transjugular liver biopsy with hepatic venous pressure gradient measurement may be used as a diagnostic tool, but should solely be applied in selected high-risk patients.[44] Reversibility of histological features is uncertain and bevacizumab seems to protect against development of NRH.[38]

Chemotherapy-associated steatohepatitis

Irinotecan is associated with chemotherapy-induced steatohepatitis with a widely ranging incidence reported in literature,[47] and steatohepatitis after irinotecan was proved to increase the risk of death from PLF.[48] Histopathological findings, prediction and prevention will be discussed below in conjunction with steatosis/steatohepatitis.

Non-alcoholic fatty liver disease and non-alcoholic steatohepatitis

The prevalence of non-alcoholic fatty liver disease (NAFLD) in the adult Western population is approximately 20–30% and around 3–5% of adults are estimated to have non-alcoholic steatohepatitis (NASH)[49]. Despite lack of absolute consensus,[50,51] steatosis and NAFLD seem to be risk factors for PLF and higher overall postoperative morbidity and mortality.[2,52,53] Rodent models show that vulnerability of the steatotic liver may be due to reduced tolerance against ischemic injury caused by decreased perfusion of the liver.[54] In addition, mitochondrial dysfunction in NAFLD results in impaired ATP synthesis, while Kupffer cell dysfunction increases reactive oxygen production which causes hepatocellular injury.[54,55] In the steatotic liver the ability of hepatocytes to regenerate after major tissue loss is impaired.[56] Multiple pathways contribute to unresponsiveness of fatty hepatocytes to regenerative stimuli, and subsequent cell cycle arrest.[57] Furthermore, cell cycle transition may be negatively affected by disturbed energy homeostasis in the fatty liver.[55] Biopsy remains the most reliable method for assessment of NAFLD but is increasingly replaced by non-invasive alternatives due to a small risk for complications and sampling errors.[58,59] Non-invasive methods consist of functional liver tests, breath tests, imaging, and biomarkers that assess steatosis and fibrosis. Most patients with NAFLD have normal liver function tests, however some have elevated ALT, AST, GGT, and/or serum ferritin. Ultrasonography is still the imaging modality of choice in patients with >33% parenchymal steatosis, but its accuracy decreases in obese patients.[60] Magnetic resonance (MR) imaging (MRI) and MR spectroscopy directly quantify fat and outperform computed tomography (CT) and ultrasonography for prediction of steatosis when fat content is >5.5%.[61] Transient elastography (TE) and MR elastography (MRE) indicate fibrosis by

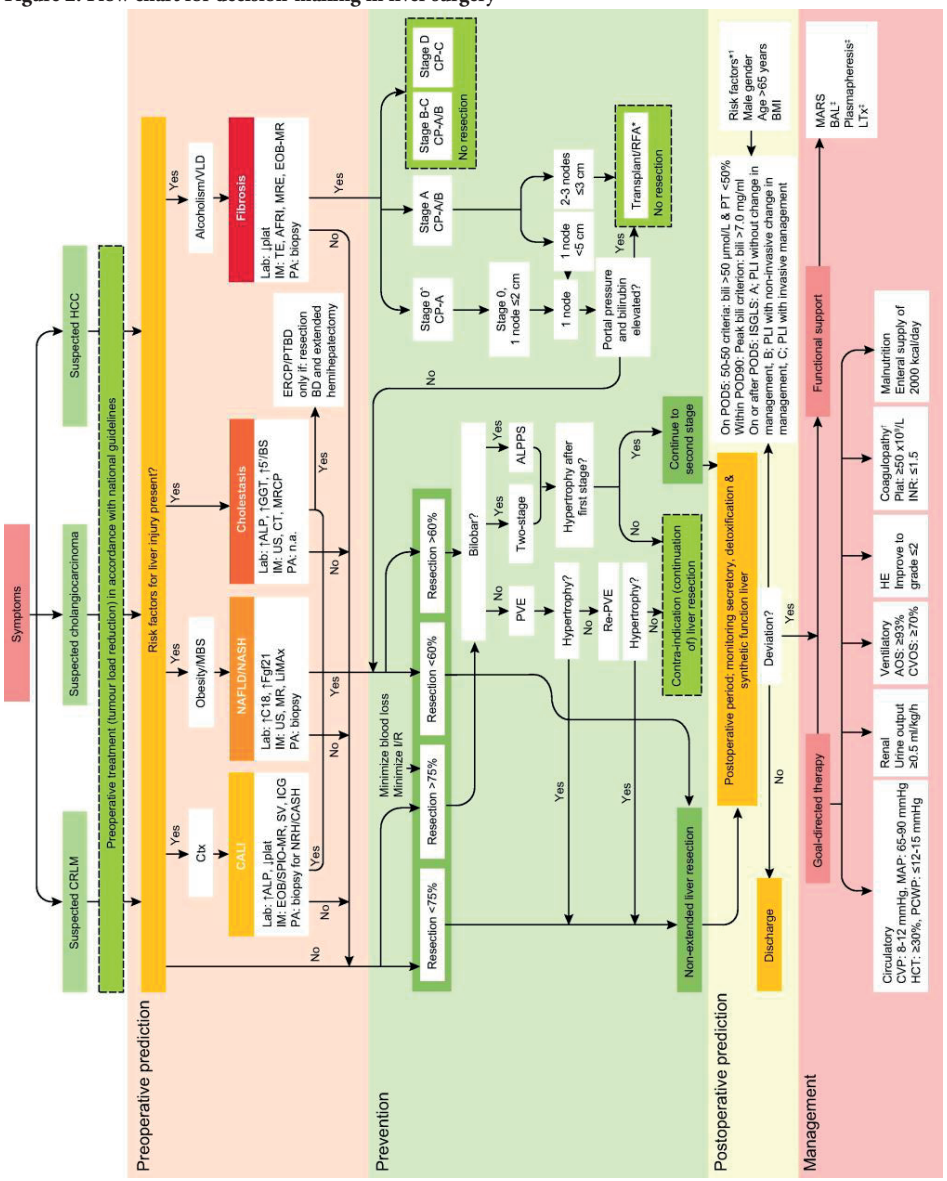
measuring liver stiffness. TE predominantly detects cirrhosis,[62] and MRE can distinguish advanced from mild fibrosis.[63] Especially TE is easily applied in clinic but its use is limited by obesity, although utilization of an XL-probe improves accuracy in obese patients.[64] Simultaneous measurement of steatosis and fibrosis can be accomplished by integration of the controlled attenuation parameter in TE or acoustic radiation force impulse in a conventional ultrasonography machine.[65,66] Serum fibroblast growth factor 21 (Fgf21) and cytokeratin 18 are biomarkers that can discriminate between NASH and NAFLD,[67,68] and NAFLD, NASH and fibrosis.[67,68] The fibrosis-4 score showed a negative predictive value of 98% for detecting patients without advanced fibrosis.[69] Other combined parameters that assess hepatic fibrosis are the APRI, FibroMeter NAFLD, NAFLD fibrosis score, and BARD score.[70–73]

Fibrosis and cirrhosis

Hepatic fibrosis is mainly present in patients undergoing partial liver resection for HCC and is mostly caused by progression of steatosis or related to chronic viral hepatitis.[74] In the past, the decreased regenerative capacity of the fibrotic liver increased the risk of PLF and caused postoperative mortality rates of around 15%.[75] Present mortality rates have declined to 0–5% due to advances in preoperative liver function assessment and strict patient selection[74] (Figure 2). Little is known about the influence of fibrosis on PLF. Regeneration of the fibrotic liver is suggested to be a progenitor cell-mediated process, in contrast to replication of existing mature hepatocytes in the non-compromised liver.[76] Animal studies indicate that impaired regeneration and subsequent hepatic dysfunction following partial liver resection are due to inefficient induction of cell cycle transition mediators, hepatocyte necrosis, and a pronounced fibrogenic response.[76,77] Enhanced bacterial translocation and decreased innate and adaptive immune system activity add to vulnerability of the fibrotic liver as shown in animal and human studies.[78] For diagnostic purposes, percutaneous biopsy is increasingly replaced by four-pass transjugular biopsy,[79] which provides the advantage of concurrent measurement of the hepatic venous pressure gradient (HVPG). Class I biomarkers (e.g., AST) reflect activity of fibrogenesis, whereas class II biomarkers (e.g., APRI) correlate with fibrosis.[80] TE is the most applied technique but shows low accuracy in patients with obesity or ascites.[81]

Both TE and acoustic radiation force impulse have high accuracy for assessment of cirrhosis.[81] Additionally, multiple combination serum tests, such as the FibroTest, Hepascore, and FibroMeter, are used with or without TE.[82] Gadolinium-enhanced MRI is promising as it showed significant signal intensity differences between patients with and without fibrosis.[83] Two preoperative parameters that directly predict development of PLF in patients with cirrhosis are an RLV-to-body weight ratio <1.4%[84] and the change in portal venous pressure.[85] Furthermore, whereas portal hypertension ought to be a contraindication for

Figure 2. Flow chart for decision-making in liver surgery



5',5'-nucleotidase; AFRI, acoustic radiation force impulse imaging; ALP, alkaline phosphatase; ALPPS, associating liver partition and portal vein ligation for staged hepatectomy; ALT, alanine transaminase; AOS, arterial oxygen saturation; AST, aspartate transaminase; BAL, bio-artificial liver; bili, total bilirubin; BD, bile duct; BMI, body mass index; BS, bile salts; C18, cytokeratin 18; CALI, chemotherapy-associated liver injury; CASH, chemotherapy-associated steatohepatitis; CP, Child-Pugh; CPV, central venous pressure; CRLM, colorectal liver metastases; CT, computed tomography; Ctx, chemotherapy; CVOS, central venous oxygen saturation; EOB, gadoxetic acid-enhanced; ERCP, endoscopic retrograde cholangiopancreatography; Fgf21, fibroblast growth factor 21; GGT, gamma-glutamyl transferase; HCC, hepatocellular carcinoma; HCT, haematocrit; HE, hepatic encephalopathy; I/R, ischemia-reperfusion injury; ICG, indocyanine green clearance; IM, imaging; INR, international normalized ratio; ISGLS, International Study Group of Liver Surgery; Lab, laboratory findings; Ltx, liver transplantation; MAP, mean arterial pressure; MARS, molecular absorbent recirculation system; MBS, metabolic syndrome; MR, magnetic resonance imaging; MRCP, magnetic reso-

nance cholangiopancreatography; MRE, magnetic resonance elastography; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NRH, nodular regenerative hyperplasia; PA, pathology; PCWP, pulmonary capillary wedge pressure; plat, platelets; PLI, postoperative liver insufficiency; POD, postoperative day; PT, prothrombin time; PTBD, percutaneous transhepatic biliary drainage; PVE, portal vein embolization; Re-PVE, recurrent portal vein embolization; RFA, radiofrequency ablation; SPIO, superparamagnetic iron oxide-enhanced; SV, splenic volume; TE, transient elastography; US, ultrasonography. ^Barcelona clinic criteria *Resection criteria are expanded and presumably differ between centres; †in case of bleeding; ‡only tested pre-clinically or in acute liver failure/acute-on-chronic liver failure

hepatic resection in patients with HCC, a recent study on the relationship between the HVPG and the development of PLF found that even in patients with a pressure gradient ≥10 mmHg, one-quarter of the patients experienced an uneventful postoperative course.[86]

Cholestasis

Obstructive cholestasis is characterized by retention of biliary constituents and a ductular reaction, and upon longer duration by hepatocyte degeneration, bile salt stasis, and progression of the ductular reaction to biliary fibrosis.[87] Patients with perihilar CCA often present with jaundice, weight loss, and cholangitis, whereas intrahepatic CCA is frequently associated with a silent clinical course and general symptoms such as malaise and loss of appetite resulting in late detection.[88] After extensive resection for perihilar CCA, PLF is seen in up to 30% of patients and mortality occurs in around 8–12% of patients,[89,90] possibly due to a combination of cholangitis and a small RLV.[91] A complication rate of up to 38% is reported after surgical removal of intrahepatic CCA, with few patients developing PLF and a mortality rate of approximately 1%.[92] Animal studies suggest that biliary dilatation caused by distal obstruction compresses the portal triad resulting in a decreased portal flow with subsequent compensatory increased arterial flow in combination with portosystemic shunting (reviewed in reference 93). Additionally, the interrupted enterohepatic circulation, lower expression of proliferative mediators in the priming and early phase of regeneration, and toxic bile-associated hepatocyte apoptosis, add to defective regeneration after partial resection of the obstructed liver in rodents.[93] A significant suppression of mitotic indices and lower hepatic weight gain after partial hepatectomy is observed in cholestatic rats.[94] Furthermore, animal studies provided evidence for enhanced susceptibility to post-ischemic reperfusion injury in cholestatic rats.[95] The detrimental role of Kupffer cells in cholestatic injury is demonstrated by amelioration of injury in bile duct-ligated mice with prior depletion of Kupffer cells.[96] Moreover, an excessive inflammatory response through pro-inflammatory cytokine production led to deterioration of hepatic function after bile duct ligation, resulting in enhanced susceptibility to infection.[97] Jaundiced patients undergoing laparotomy additionally showed significantly more bacterial translocation.[98] This is in line with the high clinical incidence of postoperative infectious complications in cholestatic patients undergoing partial hepatectomy.[90] Obstructive cholestasis is biochemically characterized by elevated serum bilirubin, ALP and GGT levels.[99] Inflammatory parameters are elevated in case of acute cholangitis.[100] Imaging of

cholestatic parenchyma using ultrasonography, CT or MR cholangiopancreatography is not focused on assessing quality but on detection of dilated intrahepatic bile ducts.

ASSESSMENT OF LIVER VOLUME AND FUNCTION

Both assessment of liver volume and function is mandatory to predict postoperative functional reserve. Methods for measurement of future RLV range from 2D volumetry on computed tomography, to perioperative 3D modelling. Computational software allows manual or automatic delineation of the liver on all CT or MRI sections, thereby allowing calculation of liver volume.[101,102] Liver function can be estimated by preoperative biochemistry, breath tests and imaging. Hepatic secretory (bilirubin), synthetic (INR) and detoxifying (ammonia) functions and liver damage (ALT, AST) are evaluated by clinical chemistry. Metabolic liver function testing can be performed with the LiMAx test and the indocyanine green clearance rate (ICGR-15).[11,12] The LiMAx test measures metabolism of intravenously injected 13C-labeled methacetin in exhaled breath. Imaging techniques used in clinic include 99mTc-labeled galactosyl serum albumin (GSA) liver scintigraphy, 99mTc-mebrofenin hepatobiliary scintigraphy with single-photon emission computed tomography (SPECT), and gadolinium enhanced MRI using gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid (Gd-EOB-DTPA).[103–105] Impaired enhancement of labelled contrast indicates decreased hepatic uptake and reflects compromised liver quality. Liver enhancement in gadolinium-enhanced MRI shows good correlation with regional liver function and offers the advantage of simultaneous diagnostic evaluation and functional assessment.[104]

PREVENTION OF POSTRESECTIONAL LIVER FAILURE

Prevention of PLF consists of four principles: optimizing preoperative liver function, enlarging RLV, limiting hepatic haemodynamic disbalance and providing optimal perioperative care.

(Pre-)clinical methods of preoperative liver optimization

The liver of patients with SOS is in a prothrombotic state as reflected by upregulation of plasminogen activator inhibitor-1, Von Willebrand factor and factor X.[106] The fibrinolytic agent defibrotide is administered in bone marrow transplant recipients for treatment of SOS,[107] and might be beneficial in chemotherapy-related SOS as well. Anti-platelet therapy such as aspirin seems to protect against oxaliplatin-induced SOS in patients.[108]

Oxaliplatin is conjugated to glutathione and subsequently excreted from the cell, which is the probable cause of reduced hepatic glutathione levels seen in SOS.[106] Supplementation of antioxidant therapy (hydroxyanisole) or flavonoids reduced the severity of sinusoidal injury in rodents.[106,109] This effect has not yet been confirmed in humans. Chemotherapy-free interval prior to liver resection may reverse SOS, as suggested by a longer period since the last cycle of chemotherapy in patients without histological evidence of SD at the time of liver resection.[32] On the other hand, hepatic sinusoidal lesions and even progression of fibrosis are reported up to several months after cessation of chemotherapy.[31] Portal hypertension can be diminished by perioperative splenic artery ligation in patients with severe NRH and portal hypertension, and might decrease postoperative morbidity.[110] Liver steatosis can be reduced by a preoperative very-low calorie diet, as has been shown in potential liver transplant donors.[111] Less steatosis and steatohepatitis was observed in patients with one week of calorie restriction prior to resection for benign or malignant liver disease, compared to ad lib fed patients.[112] However, despite less intraoperative blood loss in the diet group, no effect was seen on postoperative complications in this patient group. Optimization of liver function in patients with cirrhosis has not yet been attempted, however, platelet infusion may be an option. Thrombocytopenia in cirrhosis may be caused by a decrease in (hepatic) thrombopoietin production and systemic removal of platelets in the spleen.[113] Platelets have a stimulatory effect on liver regeneration,[114] and platelet infusion might provide an option for preoperative optimization. The preventive role of preoperative biliary drainage in obstructive cholestasis is uncertain. Internal (stenting via endoscopic retrograde cholangiopancreatography, ERCP) or external (percutaneous transhepatic biliary drainage, PTBD) drainage in pancreatic head cancer patients has been shown to have no benefits on surgical outcome and induced drainage-related complications,[115] whereas its role in proximal malignant bile duct obstruction is inconclusive. Preoperative improved secretory liver function, improved postoperative liver regeneration, and a reduction of mortality after right hemihepatectomy were reported,[116] but this could not be reproduced by others.[117,118] Drainage-related complications such as cholangitis and haemorrhage are seen in up to 33% of patients.[116] Especially infectious complications are more frequent after ERCP stenting,[119] whereas PTBD causes interruption of the enterohepatic cycle and impairment of liver regeneration.[15] Bile salt reinfusion during PTBD had beneficial effects on postoperative liver function.[120]

Enlarging of future remnant liver volume

Hypertrophy-inducing procedures and surgical adaptations should be performed if the RLV is expected to be <25% in patients without liver disease and <35–40% in patients with impaired liver function.[8] In general, portal vein embolization (PVE, Figure 3) enlarges the RLV with approximately 35–40% and improves eligibility for hepatectomy by 20%.[1] In less than 5% of patients the hypertrophic response following PVE is in-

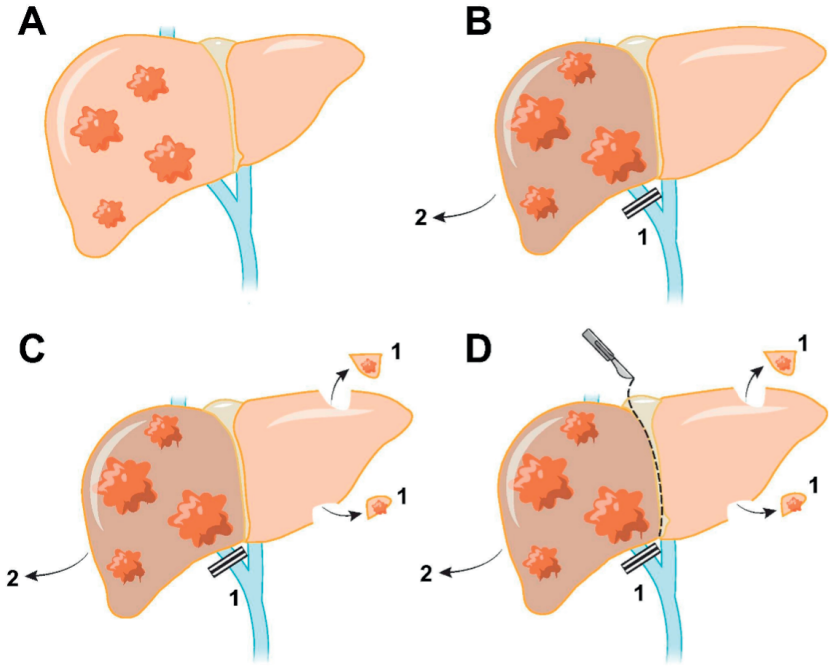
adequate.[121] Major PVE-related complications occur in approximately 2.5% of patients and include intraabdominal abscess, liver hematoma, and backflow of embolization material.[121] A recent meta-analysis comparing PVE with ligation of the portal vein (PVL) showed comparable preoperative hypertrophic responses and postoperative morbidity.[122] New developments exist of polyvinyl alcohol particles with plugs or coils as embolizing materials, and have resulted in lower recanalization rates, enhanced hypertrophy, and a decreased occurrence of PLF.[123] Disease progression after PVE occurs in up to 66% of patients, and is likely due to increased arterial flow to the embolized lobe and/or waiting period to surgery.[124] The interval between PVE and surgery should therefore be as short as possible but not less than 2–3 weeks.[124] Post-PVE chemotherapy before resection may halt disease progression without affecting subsequent liver regeneration.[125] PVE is commonly performed after the administration of chemotherapy.[108] Evidence for the influence of CALI on post-PVE hypertrophy is conflicting. Whereas SD seems to have a clear inhibitory effect on hypertrophy,[126] chemotherapy has no effect on liver regrowth.[121,127] Moreover, patients with NASH show a trend towards less post-PVE liver volume gain compared to patients with normal liver function.[126] Although robust evidence is lacking,[121] cholestasis appears to have no negative impact on hypertrophy after PVE. After right hepatectomy in patients with chronic liver disease, PLF developed in 50% of patients without PVE vs. 7.1% in patients with PVE.[128] Impaired hypertrophy after technically successful PVE in patients with chronic liver disease is a contraindication for major resection.[128] The two-staged hepatectomy is an excellent method to increase RLV and consequently achieve curation in patients with bilobar tumours, who are not deemed resectable in one attempt. PVL concurrent with two-stage hepatectomy resulted in an RLV gain of about 40% after eight weeks. This strategy is advised in case of an RLV after the first stage of <25–30% and <40% in patients without and with chronic liver disease, respectively. Liver cirrhosis is a contraindication for the two-staged procedure. The recently developed associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) procedure is based on the same principle as two-stage hepatectomy, albeit that during the first stage in ALPPS the liver is split in situ combined with portal vein ligation, and the second stage consists of removal of the ligated lobe 7–14 days later.[129] An astonishing average hypertrophy rate of 80% can be achieved with this procedure, creating a curative opportunity for initially non-resectable patients who have insufficient hypertrophy on PVE.[130] However, the high morbidity and mortality rates up to 28% and 9% respectively restrict the use of ALPPS to fit patients under the age of 60 years.[130] The second stage of ALPPS should probably be (temporarily) abolished in patients who develop major complications after the first stage.[131] Although ALPPS is currently performed in all liver backgrounds, histological changes comprising fibrosis, steatosis, and chemotherapy-related alterations resulted in lower hypertrophy rates.[130] The ALPPS procedure resulted in a quadrupled mortality rate, doubled median hospital stay, and doubled risk for PLF in patients with intermediate

stage HCC[132] and should therefore only be applied in HCC patients with low-grade fibrosis. ALPPS should not, or with great caution, be applied in patients with perihilar and intrahepatic CCA due to the (already) high postoperative mortality rate in this patient category.[130,133] Modifications such as monosegment ALPPS, in which only one instead of two or more Couinaud segments remain, showed promising results in a small cohort of 12 patients, with a PLF rate of 33% but without mortality.[134]

Limiting hepatic haemodynamic imbalance

Splenectomy and splenic artery ligation can be effective strategies that limit the postresectional increase in portal blood flow and pressure, by activating the hepatic arterial buffer response. These procedures resulted in increased arterial inflow, and enhanced liver regeneration and liver function after (extended) partial hepatectomy in rodent models.[135] Furthermore, in animal models of partial hepatectomy and small-for-size liver grafts, the administration of terlipressin and somatostatin seemed to reduce postresectional portal hyperperfusion and increase regenerative parameters.[136–138]

Figure 3. Visualization of pre- or perioperative interventions and their effect on liver remnant volume



(A) Malignant liver disease **(B)** Embolization/ligation of the right portal branch (1) results in atrophy of the right hemi-liver and compensatory growth of the left hemi-liver, which can be removed when appropriate hypertrophy has been achieved (2). **(C)** Removal of tumours from the left hemi-liver and occlusion of the right portal branch (1). After 4–6 weeks, the volume of the left hemi-liver is increased, and the right hemi-liver can be removed (2). **(D)** Removal of tumours from the left hemi-liver, in situ splitting of the hemi-livers, and simultaneous ligation of the right portal vein branch (1). After one week, augmented hypertrophy of the left hemi-liver permits removal of the right hemi-liver (2)

Providing optimal perioperative care

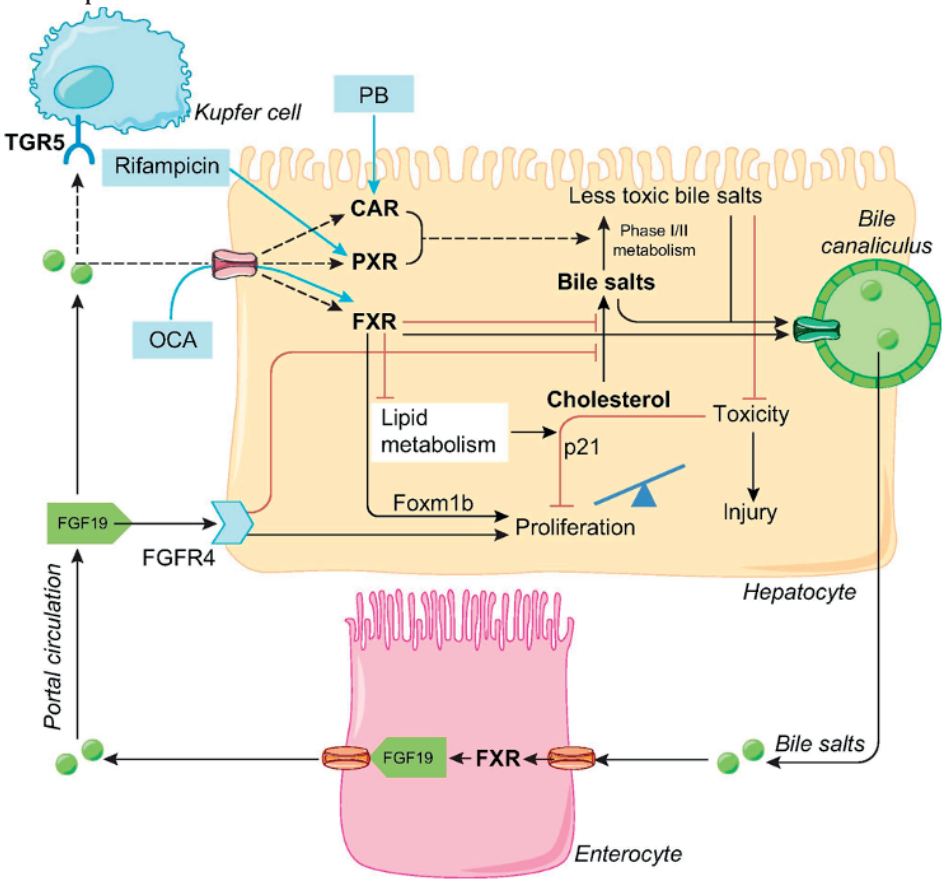
Excessive perioperative blood loss, blood transfusion, ischemia-reperfusion injury, and hepatic manipulation predispose to PLF. Blood transfusion enhances postoperative morbidity and tumour recurrence presumably via a transfusion-related inflammatory response.[139] A recent metanalysis confirmed that hepatic preconditioning (i.e., intermittent vascular inflow occlusion) results in less intraoperative blood loss and a shorter operating time in comparison to hepatectomy alone, but without improved postoperative outcome.[140] Prolonged clamping should nonetheless be avoided since ischemia-reperfusion injury has been shown to induce severe hepatic damage.[141] Hepatic manipulation per se elicits an inflammatory response.[142] Methods to minimize mobilization of the liver include laparoscopic surgery and the hanging method.[143] Laparoscopic resection of HCCs reduced the incidence of PLF compared to open surgery.[144] Since infectious complications such as bile leakage or abdominal collections may contribute to the development of PLF and negatively affect the postresectional course, several preventive measures have been explored. Postresectional primary placement of abdominal drains proved not to be beneficial after major liver resection and is even associated with increased rates of complications such as bile leakage and PLF.[145,146] Multiple human studies focused on either pre- or postresectional antibiotic prophylaxis, without evidence for a significant effect on the rate of infectious complications.[147,148] Preoperative selective bowel contamination has been explored in rodent models, showing amelioration of parenchymal injury and increased liver regeneration after partial liver resection.[149] A meta-analysis of human transplant studies however showed no benefits on infectious complications.[150]

Regenerative interventions

Augmentation of the regenerative response after liver resection may be an option for prevention and treatment of PLF. The nuclear bile salt receptor FXR (farnesoid X receptor, Figure 4) may be an attractive therapeutic candidate, through effects on hepatic haemodynamics, bile salt and lipid homeostasis, hepatic inflammation, and hepatocellular proliferation.[18,151–153] Being the key regulator of hepatic bile salt homeostasis, genetic disruption of Fxr in mice resulted in mortality and delayed liver regeneration after partial hepatectomy. Conversely, activation of Fxr by its endogenous ligands (i.e., bile salts) or synthetic agonists enhanced liver regeneration in hepatectomized mice. Furthermore, the FXR-regulated enterokine FGF19 reduced mortality in an acute liver failure mouse model.[17] FXR agonists undergo current clinical evaluation, and already showed efficacy in halting fibrotic progression in NASH patients.[154] Bearing in mind that tight control of bile salt homeostasis and hepatic inflammatory tone is warranted to allow normal progression of liver regeneration, targeting of the membrane bile salt receptor TGR5 may be considered in PLF. In the liver, TGR5 is expressed in liver endothelial cells, cholangiocytes, and Kupffer cells.[155,156] Tgr5 enhances bile salt elimination in urine, reduces bile

salt hydrophobicity and prevents excessive cytokine production by Kupffer cells, in case of bile salt overload.[14]

Figure 4. Targeting of bile salt receptors may improve liver regeneration after partial hepatectomy through direct trophic and bile salt homeostatic effects



Control of bile salt homeostasis ensures proper progression of postresectional liver regeneration. Bile salt receptor FXR in small intestine and liver exerts homeostatic control by regulating import, synthesis, conjugation (i.e., N-amidation) and export of bile salts. Moreover, bile salt signalling via FXR results in induction of genes engaged in cell cycle control (e.g., Foxm1b). On the other hand, hepatic bile salt overload gives rise to liver injury. Bile salt toxicity may be reduced by stimulation of phase I/II metabolism and phase III efflux via agonistic activation of nuclear receptors PXR and CAR. An excessive inflammatory response of Kupffer cells may be dampened by TGR5 agonism. OCA, obeticholic acid (FXR agonist); PB, phenobarbital (CAR activator); FGFR4, fibroblast growth factor receptor 4

Other nuclear receptors that play a direct role in liver regeneration, and have the potential to reduce intrahepatic bile salt toxicity by promoting phase I/II metabolism, are the pregnane X receptor and constitutive androstane receptor.[157,158] A recent study showed that the pregnane X receptor agonist rifampicin improved hyperbilirubinemia and clinical status in patients with persistent hepatocellular failure, including one patient with PLF.[159]

Despite in-depth knowledge of the processes controlled by the above (nuclear) receptors, their roles in liver regeneration and implication in PLF have only been studied in animal models. A recently discovered negative regulator of liver regeneration after partial hepatectomy, viz. thrombospondin-1,[160] might be a target to accelerate regeneration by antagonizing its action through administration of leucine-serine-lysine-leucine (LSKL) peptide.[161] Likewise, usefulness of colony stimulating factor to accelerate postresectional restoration of phagocytic capacity in the human setting is worth exploring.[162] Given the multifactorial origin of PLF strategies that simultaneously target multiple aetiological pillars may prove most effective. Transplantation of hepatocytes and other cell types have been moderately successful in several liver diseases in terms of spontaneous recovery or bridging to orthotopic liver transplantation (see reference 163 for a review), and might be of interest for preoperative optimization of liver parenchyma or management of PLF. Moreover, intrahepatic or extrahepatic (scaffold-bound) introduction of induced pluripotent stem cells (iPSC), iPSC-derived or Lgr5+ stem cell-derived organoids, and cultured hepatocytes are extensively studied in a pre-clinical setting and might offer advanced possibilities for pre- or postoperative liver repopulation.[164–168]

MANAGEMENT OF POSTOPERATIVE LIVER FAILURE

Due to the lack of randomized controlled trials with PLF as primary outcome measure, almost no treatments for acute and acute-on-chronic liver failure have been validated for PLF. When PLF is detected after resection in (non-)compromised liver, goal directed therapy and functional support can be offered (Figure 2).

Goal-directed therapy

PLF is frequently accompanied by multi-organ dysfunction, requiring a systemic treatment approach.[169] Goal-directed therapy focuses on support of circulatory, ventilatory, and renal function in combination with treatment of hepatic encephalopathy, coagulopathy and malnutrition as reviewed elsewhere.[1]

Functional support

Molecular absorbent recirculation system, an extracorporeal artificial liver support device that reduces liver failure-induced toxicity by facilitating exchange of albumin-bound and water-soluble toxins from plasma, is applicable as treatment for PLF.[170] In addition, extracorporeal bio-artificial liver devices fulfil functions of the liver (including synthetic and immunological) by separation and passage of blood plasma through a reactor containing layers of animal or human hepatocytes.[171] The recently developed University College London-Liver Dialysis Device extracts albumin by hemofiltration and removes certain

endotoxins by hemoperfusion, in combination with human albumin infusion.[172] Unfortunately, although the latter two devices show survival benefits, they have thus far been tested only in a pre-clinical setting. Furthermore, promising treatment modalities that focus on extracorporeal high-flux haemodialysis in combination with albumin dialysis (Prometheus), and patient plasma replacement with fresh frozen plasma (high-volume plasmapheresis), have been tested almost exclusively as treatment for acute and acute-on-chronic liver failure with sparse (underpowered) data on its use in the context of liver failure after hepatic resection.[173,174]

Rescue and elective liver transplantation

The limited data on rescue liver transplantation in patients with PLF showed a 5-year overall survival of 40%,[175] however appropriate criteria for patient selection are lacking. Hence, rescue liver transplantation is barely applied nowadays. Moreover, rescue liver transplantation should not be performed if the patient was not eligible for transplantation before partial hepatectomy.

CONCLUSION

The incidence of liver failure after surgical resection is relatively low. This is accomplished to a large extent by (I) better insight into the aetiology of PLF and liver regeneration, (II) new imaging techniques and biochemical tests for preoperative assessment of liver quality, (III) highly effective preventive measures, and (IV) improved perioperative care. Due to the low event rate, prospective studies with PLF as primary endpoint are nearly unachievable,[176] and most evidence is based on retrospective cohort studies. Furthermore, a uniform definition and outcome set are lacking, but imperative to compare different cohorts.[177] In view of the current increase of extensive resections in a compromised liver background, the development of universal prediction models, more advanced surgical techniques, and efficient preventive measures become particularly important to obtain curability in these challenging patients. Global collaborations and registrations such as seen in the EASL-CLIF consortium (acute-on-chronic liver failure)[130] or the ALPPS-registry[178] seem the only manner to obtain the required number of events for robust evidence on risk factors, prediction models and interventions.

REFERENCES

1. van den Broek MA, Olde Damink SW, Dejong CH, Lang H, Malago M, Jalan R, et al. Liver failure after partial hepatic resection: definition, pathophysiology, risk factors and treatment. *Liver Int* 2008; **28**:767–780.
2. Schindl MJ, Redhead DN, Fearon KC, Garden OJ, Wigmore SJ, Edinburgh Liver S, et al. The value of residual liver volume as a predictor of hepatic dysfunction and infection after major liver resection. *Gut* 2005; **54**:289–296.
3. Balzan S, Belghiti J, Farges O, Ogata S, Sauvanet A, Delefosse D, et al. The "50-50 criteria" on post-operative day 5: an accurate predictor of liver failure and death after hepatectomy. *Ann Surg* 2005; **242**:824–828.
4. Mullen JT, Ribero D, Reddy SK, Donadon M, Zorzi D, Gautam S, et al. Hepatic insufficiency and mortality in 1,059 noncirrhotic patients undergoing major hepatectomy. *J Am Coll Surg* 2007; **204**:854–862.
5. Rahbari NN, Garden OJ, Padbury R, Brooke-Smith M, Crawford M, Adam R, et al. Posthepatectomy liver failure: a definition and grading by the International Study Group of Liver Surgery (ISGLS). *Surgery* 2011; **149**:713–724.
6. Fausto N, Campbell JS, Riehle KJ. Liver regeneration. *J Hepatol* 2012; **57**:692–694.
7. Michalopoulos GK. Principles of liver regeneration and growth homeostasis. *Compr Physiol* 2013; *3*:485–513.
8. Vauthey JN, Abbott DE. Commentary on "Feasibility study of two-stage hepatectomy for bilobar liver metastases". *Am J Surg* 2012; **203**:698–699.
9. Eipel C, Abshagen K, Vollmar B. Regulation of hepatic blood flow: the hepatic arterial buffer response revisited. *World J Gastroenterol* 2010; **16**:6046–6057.
10. Allard MA, Adam R, Bucur PO, Termos S, Cunha AS, Bismuth H, et al. Posthepatectomy portal vein pressure predicts liver failure and mortality after major liver resection on noncirrhotic liver. *Ann Surg* 2013; **258**:822–829.
11. Golriz M, Majlesara A, El Sakka S, Ashrafi M, Arwin J, Fard N, et al. Small for Size and Flow (SFSF) syndrome: an alternative description for posthepatectomy liver failure. Clin Res Hepatol *Gastroenterol* 2016; **40**:267–275.
12. Erlinger S, Arias IM, Dhumeaux D. Inherited disorders of bilirubin transport and conjugation: new insights into molecular mechanisms and consequences. *Gastroenterol* 2014; **146**:1625–1638.
13. Doignon I, Julien B, Serriere-Lanneau V, Garcin I, Alonso G, Nicou A, et al. Immediate neuroendocrine signaling after partial hepatectomy through acute portal hyperpressure and cholestasis. *J Hepatol* 2011; **54**:481–488.
14. Pean N, Doignon I, Garcin I, Besnard A, Julien B, Liu B, et al. The receptor TGR5 protects the liver from bile acid overload during liver regeneration in mice. *Hepatology* 2013; **58**:1451–1460.
15. Huang W, Ma K, Zhang J, Qatanani M, Cuvillier J, Liu J, et al. Nuclear receptor-dependent bile acid signaling is required for normal liver regeneration. *Science* 2006; **312**:233–236.
16. Perez MJ, Briz O. Bile-acid-induced cell injury and protection. *World J Gastroenterol* 2009; **15**:1677–1689.
17. Uriarte I, Fernandez-Barrena MG, Monte MJ, Latasa MU, Chang HC, Carotti S, et al. Identification of fibroblast growth factor 15 as a novel mediator of liver regeneration and its application in the prevention of post-resection liver failure in mice. *Gut* 2013; **62**:899–910.
18. Fan M, Wang X, Xu G, Yan Q, Huang W. Bile acid signaling and liver regeneration. Biochim *Biophys Acta* 2015; **1849**:196–200.

19. Fausto N, Campbell JS, Riehle KJ. Liver regeneration. *Hepatology* 2006;**43**: S45–S53.
20. Chen Y, Sun R. Toll-like receptors in acute liver injury and regeneration. *Int Immunopharmacol* 2011; **11**:1433–1441.
21. Takeishi T, Hirano K, Kobayashi T, Hasegawa G, Hatakeyama K, Naito M. The role of Kupffer cells in liver regeneration. *Arch Histol Cytol* 1999; **62**:413–422.
22. Prins HA, Meijer C, Boelens PG, Diks J, Holtz R, Masson S, et al. Kupffer cell depleted rats have a diminished acute-phase response following major liver resection. *Shock* 2004; **21**:561–565.
23. Schindl MJ, Millar AM, Redhead DN, Fearon KC, Ross JA, Dejong CH, et al. The adaptive response of the reticuloendothelial system to major liver resection in humans. *Ann Surg* 2006; **243**:507–514.
24. Liu HX, Keane R, Sheng L, Wan YJ. Implications of microbiota and bile acid in liver injury and regeneration. *J Hepatol* 2015; **63**:1502–1510.
25. Liu HX, Rocha CS, Dandekar S, Yvonne Wan YJ. Functional analysis of the relationship between intestinal microbiota and the expression of hepatic genes and pathways during the course of liver regeneration. *J Hepatol* 2016; **64**:641–650.
26. Joyce SA, MacSharry J, Casey PG, Kinsella M, Murphy EF, Shanahan F, et al. Regulation of host weight gain and lipid metabolism by bacterial bile acid modification in the gut. *Proc Natl Acad Sci U S A* 2014; **111**:7421–7426.
27. Sayin SI, Wahlstrom A, Felin J, Jantti S, Marschall HU, Bamberg K, et al. Gut microbiota regulates bile acid metabolism by reducing the levels of taurobeta-muricholic acid, a naturally occurring FXR antagonist. *Cell Metab* 2013; **17**:225–235.
28. Liu HX, Hu Y, Wan YY. Microbiota and bile acid profiles in retinoic acidprimed mice that exhibit accelerated liver regeneration. *Oncotarget* 2016; **7**:1096–1106.
29. Cornell RP. Gut-derived endotoxin elicits hepatotrophic factor secretion for liver regeneration. *Am J Physiol* 1985;**249**: R551–R562.
30. Cornell RP. Restriction of gut-derived endotoxin impairs DNA synthesis for liver regeneration. *Am J Physiol* 1985;**249**: R563–R569.
31. Rubbia-Brandt L, Audard V, Sartoretti P, Roth AD, Brezault C, Le Charpentier M, et al. Severe hepatic sinusoidal obstruction associated with oxaliplatin-based chemotherapy in patients with metastatic colorectal cancer. *Ann Oncol* 2004; **15**:460–466.
32. Nakano H, Oussoultzoglou E, Rosso E, Casnedi S, Chenard-Neu MP, Dufour P, et al. Sinusoidal injury increases morbidity after major hepatectomy in patients with colorectal liver metastases receiving preoperative chemotherapy. *Ann Surg* 2008; **247**:118–124.
33. Soubrane O, Brouquet A, Zalinski S, Terris B, Brezault C, Mallet V, et al. Predicting high grade lesions of sinusoidal obstruction syndrome related to oxaliplatin-based chemotherapy for colorectal liver metastases: correlation with post-hepatectomy outcome. *Ann Surg* 2010; **251**:454–460.
34. Vigano L, Ravarino N, Ferrero A, Motta M, Torchio B, Capussotti L. Prospective evaluation of accuracy of liver biopsy findings in the identification of chemotherapy-associated liver injuries. *Arch Surg* 2012; **147**:1085–1091.
35. Schiffer E, Frossard JL, Rubbia-Brandt L, Mentha G, Pastor CM. Hepatic regeneration is decreased in a rat model of sinusoidal obstruction syndrome. *J Surg Oncol* 2009; **99**:439–446.
36. Hubert C, Dahrenmoller C, Marique L, Jabbour N, Gianello P, Leclercq I. Hepatic regeneration in a rat model is impaired by chemotherapy agents used in metastatic colorectal cancer. *Eur J Surg Oncol* 2015; **41**: 1471–1478.
37. Ribero D, Wang H, Donadon M, Zorzi D, Thomas MB, Eng C, et al. Bevacizumab improves pathologic response and protects against hepatic injury in patients treated with oxaliplatin-based chemotherapy for colorectal liver metastases. *Cancer* 2007; **110**:2761–2767.

38. Rubbia-Brandt L, Lauwers GY, Wang H, Majno PE, Tanabe K, Zhu AX, et al. Sinusoidal obstruction syndrome and nodular regenerative hyperplasia are frequent oxaliplatin-associated liver lesions and partially prevented by bevacizumab in patients with hepatic colorectal metastasis. *Histopathology* 2010; **56**:430–439.
39. Imai K, Emi Y, Iyama KI, Beppu T, Ogata Y, Kakeji Y, et al. Splenic volume may be a useful indicator of the protective effect of bevacizumab against oxaliplatin-induced hepatic sinusoidal obstruction syndrome. *Eur J Surg Oncol* 2014; **40**:559–566.
40. Overman MJ, Maru DM, Charnsangavej C, Loyer EM, Wang H, Pathak P, et al. Oxaliplatin-mediated increase in spleen size as a biomarker for the development of hepatic sinusoidal injury. *J Clin Oncol* 2010; **28**:2549–2555.
41. Ward J, Guthrie JA, Sheridan MB, Boyes S, Smith JT, Wilson D, et al. Sinusoidal obstructive syndrome diagnosed with superparamagnetic iron oxide enhanced magnetic resonance imaging in patients with chemotherapy treated colorectal liver metastases. *J Clin Oncol* 2008; **26**:4304–4310.
42. Shin NY, Kim MJ, Lim JS, Park MS, Chung YE, Choi JY, et al. Accuracy of gadoxetic acid-enhanced magnetic resonance imaging for the diagnosis of sinusoidal obstruction syndrome in patients with chemotherapy-treated colorectal liver metastases. *Eur Radiol* 2012; **22**:864–871.
43. Wanless IR. Micronodular transformation (nodular regenerative hyperplasia) of the liver: a report of 64 cases among 2,500 autopsies and a new classification of benign hepatocellular nodules. *Hepatology* 1990; **11**:787–797.
44. Vigano L, Rubbia-Brandt L, De Rosa G, Majno P, Langella S, Toso C, et al. Nodular regenerative hyperplasia in patients undergoing liver resection for colorectal metastases after chemotherapy: risk factors, preoperative assessment and clinical impact. *Ann Surg Oncol* 2015; **22**:4149–4157.
45. van den Broek MA, Olde Damink SW, Driessen A, Dejong CH, Bemelmans MH. Nodular regenerative hyperplasia secondary to neoadjuvant chemotherapy for colorectal liver metastases. *Case Rep Med* 2009.
46. Wicherts DA, de Haas RJ, Sebagh M, Ciacio O, Levi F, Paule B, et al. Regenerative nodular hyperplasia of the liver related to chemotherapy: impact on outcome of liver surgery for colorectal metastases. *Ann Surg Oncol* 2011; **18**:659–669.
47. Robinson SM, Wilson CH, Burt AD, Manas DM, White SA. Chemotherapyassociated liver injury in patients with colorectal liver metastases: a systematic review and meta-analysis. *Ann Surg Oncol* 2012; **19**:4287–4299.
48. Vauthey JN, Pawlik TM, Ribero D, Wu TT, Zorzi D, Hoff PM, et al. Chemotherapy regimen predicts steatohepatitis and an increase in 90-day mortality after surgery for hepatic colorectal metastases. *J Clin Oncol* 2006; **24**:2065–2072.
49. Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther* 2011; **34**:274–285.
50. Little SA, Jarnagin WR, DeMatteo RP, Blumgart LH, Fong Y. Diabetes is associated with increased perioperative mortality but equivalent long-term outcome after hepatic resection for colorectal cancer. *J Gastrointest Surg* 2002; **6**:88–94.
51. Newberry EP, Kennedy SM, Xie Y, Luo J, Stanley SE, Semenkovich CF, et al. Altered hepatic triglyceride content after partial hepatectomy without impaired liver regeneration in multiple murine genetic models. *Hepatology* 2008; **48**:1097–1105.
52. de Meijer VE, Kalish BT, Puder M, Ijzermans JN. Systematic review and metaanalysis of steatosis as a risk factor in major hepatic resection. *Br J Surg* 2010; **97**:1331–1339.

53. Reddy SK, Marsh JW, Varley PR, Mock BK, Chopra KB, Geller DA, et al. Underlying steatohepatitis, but not simple hepatic steatosis, increases morbidity after liver resection: a case-control study. *Hepatology* 2012; **56**:2221–2230.
54. Tashiro H, Kuroda S, Mikuriya Y, Ohdan H. Ischemia-reperfusion injury in patients with fatty liver and the clinical impact of steatotic liver on hepatic surgery. *Surg Today* 2014; **44**:1611–1625.
55. Vetelainen R, van Vliet A, Gouma DJ, van Gulik TM. Steatosis as a risk factor in liver surgery. *Ann Surg* 2007; **245**:20–30.
56. Selzner M, Clavien PA. Failure of regeneration of the steatotic rat liver: disruption at two different levels in the regeneration pathway. *Hepatology* 2000; **31**:35–42.
57. Yang SQ, Lin HZ, Mandal AK, Huang J, Diehl AM. Disrupted signaling and inhibited regeneration in obese mice with fatty livers: implications for nonalcoholic fatty liver disease pathophysiology. *Hepatology* 2001; **34**:694–706.
58. Myers RP, Fong A, Shaheen AA. Utilization rates, complications and costs of percutaneous liver biopsy: a population-based study including 4275 biopsies. *Liver Int* 2008; **28**:705–712.
59. Regev A, Berho M, Jeffers LJ, Milikowski C, Molina EG, Pyrsopoulos NT, et al. Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. *Am J Gastroenterol* 2002; **97**:2614–2618.
60. de Moura Almeida A, Cotrim HP, Barbosa DB, de Athayde LG, Santos AS, Bitencourt AG, et al. Fatty liver disease in severe obese patients: diagnostic value of abdominal ultrasound. World J *Gastroenterol* 2008; **14**:1415–1418.
61. Lee SS, Park SH, Kim HJ, Kim SY, Kim MY, Kim DY, et al. Non-invasive assessment of hepatic steatosis: prospective comparison of the accuracy of imaging examinations. *J Hepatol* 2010; **52**:579–585.
62. Tsochatzis EA, Gurusamy KS, Ntaoula S, Cholongitas E, Davidson BR, Burroughs AK. Elastography for the diagnosis of severity of fibrosis in chronic liver disease: a meta-analysis of diagnostic accuracy. *J Hepatol* 2011; **54**:650–659.
63. Loomba R, Wolfson T, Ang B, Hooker J, Behling C, Peterson M, et al. Magnetic resonance elastography predicts advanced fibrosis in patients with nonalcoholic fatty liver disease: a prospective study. *Hepatology* 2014; **60**:1920–1928.
64. de Ledinghen V, Vergniol J, Foucher J, El-Hajbi F, Merrouche W, Rigalleau V. Feasibility of liver transient elastography with FibroScan using a new probe for obese patients. *Liver Int* 2010; **30**:1043–1048.
65. Shi KQ, Tang JZ, Zhu XL, Ying L, Li DW, Gao J, et al. Controlled attenuation parameter for the detection of steatosis severity in chronic liver disease: a meta-analysis of diagnostic accuracy. *J Gastroenterol Hepatol* 2014; **29**:1149–1158.
66. Palmeri ML, Wang MH, Rouze NC, Abdelmalek MF, Guy CD, Moser B, et al. Noninvasive evaluation of hepatic fibrosis using acoustic radiation forcebased shear stiffness in patients with nonalcoholic fatty liver disease. *J Hepatol* 2011; **55**:666–672.
67. Shen J, Chan HL, Wong GL, Choi PC, Chan AW, Chan HY, et al. Non-invasive diagnosis of non-alcoholic steatohepatitis by combined serum biomarkers. *J Hepatol* 2012; **56**:1363–1370.
68. Cusi K, Chang Z, Harrison S, Lomonaco R, Bril F, Orsak B, et al. Limited value of plasma cytokeratin-18 as a biomarker for NASH and fibrosis in patients with non-alcoholic fatty liver disease. *J Hepatol* 2014; **60**:167–174.
69. Sumida Y, Yoneda M, Hyogo H, Itoh Y, Ono M, Fujii H, et al. Validation of the FIB4 index in a Japanese nonalcoholic fatty liver disease population. *BMC Gastroenterol* 2012; **12**:2.
70. Harrison SA, Oliver D, Arnold HL, Gogia S, Neuschwander-Tetri BA. Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. *Gut* 2008; **57**:1441–1447.

71. Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology* 2007; **45**:846–854.
72. Cales P, Oberti F, Michalak S, Hubert-Fouchard I, Rousselet MC, Konate A, et al. A novel panel of blood markers to assess the degree of liver fibrosis. *Hepatology* 2005; **42**:1373–1381.
73. Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; **38**:518–526.
74. de Lope CR, Tremosini S, Forner A, Reig M, Bruix J. Management of HCC. J Hepatol 2012;56: S75–S87. 75. Makuuchi M, Sano K. The surgical approach to HCC: our progress and results in Japan. *Liver Transpl* 2004;**10**: S46–S52.
76. Kuramitsu K, Sverdlov DY, Liu SB, Csizmadia E, Burkly L, Schuppan D, et al. Failure of fibrotic liver regeneration in mice is linked to a severe fibrogenic response driven by hepatic progenitor cell activation. *Am J Pathol* 2013; **183**:182–194.
77. Kato A, Bamba H, Shinohara M, Yamauchi A, Ota S, Kawamoto C, et al. Relationship between expression of cyclin D1 and impaired liver regeneration observed in fibrotic or cirrhotic rats. *J Gastroenterol Hepatol* 2005; **20**:1198–1205.
78. Jalan R, Fernandez J, Wiest R, Schnabl B, Moreau R, Angeli P, et al. Bacterial infections in cirrhosis: a position statement based on the EASL Special Conference 2013. *J Hepatol* 2014; **60**:1310–1324.
79. Vibhakorn S, Cholongitas E, Kalambokis G, Manousou P, Quaglia A, Marelli L, et al. A comparison of four- vs. three-pass transjugular biopsy using a 19-G Tru-Cut needle and a randomized study using a cassette to prevent biopsy fragmentation. *Cardiovasc Intervent Radiol* 2009; **32**:508–513.
80. Wong GL, Espinosa WZ, Wong VW. Personalized management of cirrhosis by non-invasive tests of liver fibrosis. *Clin Mol Hepatol* 2015; **21**:200–211.
81. Bota S, Herkner H, Sporea I, Salzl P, Sirli R, Neghina AM, et al. Meta-analysis: ARFI elastography vs. transient elastography for the evaluation of liver fibrosis. *Liver Int* 2013; **33**:1138–1147.
82. Tsochatzis EA, Bosch J, Burroughs AK. Liver cirrhosis. *Lancet* 2014; **383**:1749–1761.
83. Verloh N, Utpatel K, Haimerl M, Zeman F, Fellner C, Fichtner-Feigl S, et al. Liver fibrosis and Gd-EOB-DTPA-enhanced MRI: a histopathologic correlation. *Sci Rep* 2015; **5**:15408.
84. Lin XJ, Yang J, Chen XB, Zhang M, Xu MQ. The critical value of remnant liver volume-to-body weight ratio to estimate posthepatectomy liver failure in cirrhotic patients. *J Surg Res* 2014; **188**:489–495.
85. Chen X, Zhai J, Cai X, Zhang Y, Wei L, Shi L, et al. Severity of portal hypertension and prediction of postoperative liver failure after liver resection in patients with Child-Pugh grade A cirrhosis. *Br J Surg* 2012; **99**:1701–1710.
86. Cucchetti A, Cescon M, Golfieri R, Piscaglia F, Renzulli M, Neri F, et al. Hepatic venous pressure gradient in the preoperative assessment of patients with resectable hepatocellular carcinoma. *J Hepatol* 2016; **64**:79–86.
87. Desmet VJ. Histopathology of cholestasis. *Verh Dtsch Ges Pathol* 1995; **79**:233–240.
88. Razumilava N, Gores GJ. Classification, diagnosis, and management of cholangiocarcinoma. *Clin Gastroenterol Hepatol* 2013; **11**:13–21.
89. Neuhaus P, Thelen A, Jonas S, Puhl G, Denecke T, Veltzke-Schlieker W, et al. Oncological superiority of hilar en bloc resection for the treatment of hilar cholangiocarcinoma. *Ann Surg Oncol* 2012; **19**:1602–1608.
90. Nuzzo G, Giuliante F, Ardito F, Giovannini I, Aldrighetti L, Belli G, et al. Improvement in perioperative and long-term outcome after surgical treatment of hilar cholangiocarcinoma: results of an Italian multicenter analysis of 440 patients. *Arch Surg* 2012; **147**:26–34.

91. Wiggers JK, Koerkamp BG, Cieslak KP, Doussot A, van Klaveren D, Allen PJ, et al. Postoperative mortality after liver resection for perihilar cholangiocarcinoma: development of a risk score and importance of biliary drainage of the future liver remnant. *J Am Coll Surg* 2016; **223**:321–331.e1.
92. Endo I, Gonen M, Yopp AC, Dalal KM, Zhou Q, Klimstra D, et al. Intrahepatic cholangiocarcinoma: rising frequency, improved survival, and determinants of outcome after resection. *Ann Surg 2008*; **248**:84–96.
93. Yokoyama Y, Nagino M, Nimura Y. Mechanism of impaired hepatic regeneration in cholestatic liver. *J Hepatobiliary Pancreat Surg* 2007; **14**:159–166.
94. Tracy Jr TF, Bailey PV, Goerke ME, Sotelo-Avila C, Weber TR. Cholestasis without cirrhosis alters regulatory liver gene expression and inhibits hepatic regeneration. *Surgery* 1991; **110**:176–182.
95. Yoshidome H, Miyazaki M, Shimizu H, Ito H, Nakagawa K, Ambiru S, et al. Obstructive jaundice impairs hepatic sinusoidal endothelial cell function and renders liver susceptible to hepatic ischemia/reperfusion. *J Hepatol* 2000; **33**:59–67.
96. Gehring S, Dickson EM, San Martin ME, van Rooijen N, Papa EF, Harty MW, et al. Kupffer cells abrogate cholestatic liver injury in mice. *Gastroenterology* 2006; **130**:810–822.
97. Morita Y, Yoshidome H, Kimura F, Shimizu H, Ohtsuka M, Takeuchi D, et al. Excessive inflammation but decreased immunological response renders liver susceptible to infection in bile duct ligated mice. *J Surg Res* 2008; **146**:262–270.
98. Kuzu MA, Kale IT, Col C, Tekeli A, Tanik A, Koksoy C. Obstructive jaundice promotes bacterial translocation in humans. *Hepatogastroenterology* 1999; **46**:2159–2164.
99. Liver EAftSot. EASL Clinical Practice Guidelines: management of cholestatic liver diseases. *J Hepatol* 2009; **51**:237–267.
100. Mosler P. Diagnosis and management of acute cholangitis. *Curr Gastroenterol Rep* 2011; **13**:166–172.
101. van der Vorst JR, van Dam RM, van Stiphout RS, van den Broek MA, Hollander IH, Kessels AG, et al. Virtual liver resection and volumetric analysis of the future liver remnant using open source image processing software. *World J Surg* 2010; **34**:2426–2433.
102. Dello SA, van Dam RM, Slangen JJ, van de Poll MC, Bemelmans MH, Greve JW, et al. Liver volumetry plug and play: do it yourself with ImageJ. *World J Surg* 2007; **31**:2215–2221.
103. Mao Y, Du S, Ba J, Li F, Yang H, Lu X, et al. Using Dynamic 99mT c-GSA SPECT/CT fusion images for hepatectomy planning and postoperative liver failure prediction. *Ann Surg Oncol* 2015; **22**:1301–1307.
104. Verloh N, Haimerl M, Zeman F, Schlabeck M, Barreiros A, Loss M, et al. Assessing liver function by liver enhancement during the hepatobiliary phase with Gd-EOB-DTPA-enhanced MRI at 3 Tesla. *Eur Radiol* 2014; **24**:1013–1019.
105. de Graaf W, van Lienden KP, van Gulik TM, Bennink RJ. (99m)Tc-mebrofenin hepatobiliary scintigraphy with SPECT for the assessment of hepatic function and liver functional volume before partial hepatectomy. *J Nucl Med* 2010; **51**:229–236.
106. Robinson SM, Mann J, Vasilaki A, Mathers J, Burt AD, Oakley F, et al. Pathogenesis of FOLFOX induced sinusoidal obstruction syndrome in a murine chemotherapy model. *J Hepatol* 2013; **59**:318–326.
107. Benimetskaya L, Wu S, Voskresenskiy AM, Echart C, Zhou JF, Shin J, et al. Angiogenesis alteration by defibrotide: implications for its mechanism of action in severe hepatic veno-occlusive disease. *Blood* 2008; **112**:4343–4352.
108. Brouquet A, Benoist S, Julie C, Penna C, Beauchet A, Rougier P, et al. Risk factors for chemotherapy-associated liver injuries: a multivariate analysis of a group of 146 patients with colorectal metastases. *Surgery* 2009; **145**:362–371.
109. Ezzat T, van den Broek MA, Davies N, Dejong CH, Bast A, Malago M, et al. The flavonoid monoHER prevents monocrotaline-induced hepatic sinusoidal injury in rats. *J Surg Oncol* 2012; **106**:72–78.

110. Schwarz L, Faitot F, Soubrane O, Scatton O. Splenic artery ligation for severe oxaliplatin induced portal hypertension: a way to improve postoperative course and allow adjuvant chemotherapy for colorectal liver metastases: Letter to editor: comment about "Nodular regenerative hyperplasia (NRH)
complicating oxaliplatin chemotherapy in patients undergoing resection of colorectal liver metastases". *Eur J Surg Oncol* 2014; **40**:787–788.
111. Doyle A, Dillon J, MacArthur M, Marquez M, Smith R, Grant D, et al. Treatment with optifast reduces hepatic steatosis and safely increases candidacy rates for live donor liver transplantation. The AASLD Liver Meeting, 2015.
112. Reeves JG, Suriawinata AA, Ng DP, Holubar SD, Mills JB, Barth Jr RJ. Shortterm preoperative diet modification reduces steatosis and blood loss in patients undergoing liver resection. *Surgery* 2013; **154**:1031–1037.
113. Giannini E, Borro P, Botta F, Fumagalli A, Malfatti F, Podesta E, et al. Serum thrombopoietin levels are linked to liver function in untreated patients with hepatitis C virus-related chronic hepatitis. *J Hepatol* 2002; **37**:572–577.
114. Matsuo R, Nakano Y, Ohkohchi N. Platelet administration via the portal vein promotes liver regeneration in rats after 70% hepatectomy. *Ann Surg* 2011; **253**:759–763.
115. van der Gaag NA, Rauws EA, van Eijck CH, Bruno MJ, van der Harst E, Kubben FJ, et al. Preoperative biliary drainage for cancer of the head of the pancreas. *N Engl J Med* 2010; **362**:129–137.
116. Farges O, Regimbeau JM, Fuks D, Le Treut YP, Cherqui D, Bachellier P, et al. Multicentre European study of preoperative biliary drainage for hilar cholangiocarcinoma. *Br J Surg* 2013; **100**:274–283.
117. Ferrero A, Lo Tesoriere R, Vigano L, Caggiano L, Sgotto E, Capussotti L. Preoperative biliary drainage increases infectious complications after hepatectomy for proximal bile duct tumor obstruction. *World J Surg* 2009; **33**:318–325.
118. Hochwald SN, Burke EC, Jarnagin WR, Fong Y, Blumgart LH. Association of preoperative biliary stenting with increased postoperative infectious complications in proximal cholangiocarcinoma. *Arch Surg* 1999; **134**:261–266.
119. Kloek JJ, van der Gaag NA, Aziz Y, Rauws EA, van Delden OM, Lameris JS, et al. Endoscopic and percutaneous preoperative biliary drainage in patients with suspected hilar cholangiocarcinoma. *J Gastrointest Surg* 2010; **14**:119–125.
120. Ishizawa T, Hasegawa K, Sano K, Imamura H, Kokudo N, Makuuchi M. Selective vs. total biliary drainage for obstructive jaundice caused by a hepatobiliary malignancy. *Am J Surg* 2007; **193**:149–154.
121. van Lienden KP, van den Esschert JW, de Graaf W, Bipat S, Lameris JS, van Gulik TM, et al. Portal vein embolization before liver resection: a systematic review. *Cardiovasc Intervent Radiol* 2013; **36**:25–34.
122. Pandanaboyana S, Bell R, Hidalgo E, Toogood G, Prasad KR, Bartlett A, et al. A systematic review and meta-analysis of portal vein ligation vs. portal vein embolization for elective liver resection. *Surgery* 2015; **157**:690–698.
123. Malinowski M, Geisel D, Stary V, Denecke T, Seehofer D, Jara M, et al. Portal vein embolization with plug/coils improves hepatectomy outcome. *J Surg Res* 2015; **194**:202–211.
124. Hoekstra LT, van Lienden KP, Doets A, Busch OR, Gouma DJ, van Gulik TM. Tumor progression after preoperative portal vein embolization. *Ann Surg* 2012; **256**:812–817.
125. Vetelainen R, van Vliet AK, van Gulik TM. Severe steatosis increases hepatocellular injury and impairs liver regeneration in a rat model of partial hepatectomy. *Ann Surg* 2007; **245**:44–50.
126. Narita M, Oussoultzoglou E, Chenard MP, Rosso E, Casnedi S, Pessaux P, et al. Sinusoidal obstruction syndrome compromises liver regeneration in patients undergoing two-stage hepatectomy with portal vein embolization. *Surg Today* 2011; **41**:7–17.

127. de Baere T, Teriitehau C, Deschamps F, Catherine L, Rao P, Hakime A, et al. Predictive factors for hypertrophy of the future remnant liver after selective portal vein embolization. *Ann Surg Oncol* 2010; **17**:2081–2089.
128. Farges O, Belghiti J, Kianmanesh R, Regimbeau JM, Santoro R, Vilgrain V, et al. Portal vein embolization before right hepatectomy: prospective clinical trial. *Ann Surg* 2003; **237**:208–217.
129. Schnitzbauer AA, Lang SA, Goessmann H, Nadalin S, Baumgart J, Farkas SA, et al. Right portal vein ligation combined with in situ splitting induces rapid left lateral liver lobe hypertrophy enabling 2-staged extended right hepatic resection in small-for-size settings. *Ann Surg* 2012; **255**:405–414.
130. Schadde E, Ardiles V, Robles-Campos R, Malago M, Machado M, Hernandez-Alejandro R, et al. Early survival and safety of ALPPS: first report of the International ALPPS Registry. *Ann Surg* 2014; **260**:829–836.
131. Truant S, Scatton O, Dokmak S, Regimbeau JM, Lucidi V, Laurent A, et al. Associating liver partition and portal vein ligation for staged hepatectomy (ALPPS): impact of the inter-stages course on morbimortality and implications for management. *Eur J Surg Oncol* 2015; **41**:674–682.
132. D'Haese JG, Neumann J, Weniger M, Pratschke S, Bjornsson B, Ardiles V, et al. Should ALPPS be used for liver resection in intermediate-stage HCC? *Ann Surg Oncol* 2016; **23**:1335–1343.
133. Serenari M, Zanello M, Schadde E, Toschi E, Ratti F, Gringeri E, et al. Importance of primary indication and liver function between stages: results of a multicenter Italian audit of ALPPS 2012–2014. *HPB* 2016; **18**:419–427.
134. Schadde E, Malago M, Hernandez-Alejandro R, Li J, Abdalla E, Ardiles V, et al. Monosegment ALPPS hepatectomy: extending resectability by rapid hypertrophy. *Surgery* 2015; **157**:676–689.
135. Ren YS, Qian NS, Tang Y, Liao YH, Liu WH, Raut V, et al. Beneficial effects of splenectomy on liver regeneration in a rat model of massive hepatectomy.*Hepatobiliary Pancreat Dis Int* 2012; **11**:60–65.
136. Ren Z, Xu Y, Zhu S. The effect of terlipressin on hepatic hemodynamics in small-for-size livers. *Hepatogastroenterology* 2012; **59**:208–211.
137. Fahrner R, Patsenker E, de Gottardi A, Stickel F, Montani M, Stroka D, et al. Elevated liver regeneration in response to pharmacological reduction of elevated portal venous pressure by terlipressin after partial hepatectomy. *Transplantation* 2014; **97**:892–900.
138. Xu X, Man K, Zheng SS, Liang TB, Lee TK, Ng KT, et al. Attenuation of acute phase shear stress by somatostatin improves small-for-size liver graft survival. *Liver Transpl* 2006; **12**:621–627.
139. Miki C, Hiro J, Ojima E, Inoue Y, Mohri Y, Kusunoki M. Perioperative allogeneic blood transfusion, the related cytokine response and long-term survival after potentially curative resection of colorectal cancer. *Clin Oncol (R Coll Radiol)* 2006; **18**:60–66.
140. Simillis C, Robertson FP, Afxentiou T, Davidson BR, Gurusamy KS. A network metaanalysis comparing perioperative outcomes of interventions aiming to decrease ischemia reperfusion injury during elective liver resection. *Surgery* 2016; **159**:1157–1169.
141. Datta G, Fuller BJ, Davidson BR. Molecular mechanisms of liver ischemia reperfusion injury: insights from transgenic knockout models. *World J Gastroenterol* 2013; **19**:1683–1698.
142. van den Broek MA, Shiri-Sverdlov R, Schreurs JJ, Bloemen JG, Bieghs V, Rensen SS, et al. Liver manipulation during liver surgery in humans is associated with hepatocellular damage and hepatic inflammation. *Liver Int* 2013; **33**:633–641.
143. Belghiti J, Guevara OA, Noun R, Saldinger PF, Kianmanesh R. Liver hanging maneuver: a safe approach to right hepatectomy without liver mobilization. *J Am Coll Surg* 2001; **193**:109–111.
144. Morise Z, Ciria R, Cherqui D, Chen KH, Belli G, Wakabayashi G. Can we expand the indications for laparoscopic liver resection? A systematic review and meta-analysis of laparoscopic liver resection

for patients with hepatocellular carcinoma and chronic liver disease. *J Hepatobiliary Pancreat Sci* 2015; **22**:342–352.

145. Squires 3rd MH, Lad NL, Fisher SB, Kooby DA, Weber SM, Brinkman A, et al. Value of primary operative drain placement after major hepatectomy: a multi-institutional analysis of 1,041 patients. *J Am Coll Surg* 2015; **220**:396–402.
146. Olthof PB, Coelen RJ, Wiggers JK, Besselink MG, Busch OR, van Gulik TM.External biliary drainage following major liver resection for perihilar cholangiocarcinoma: impact on development of liver failure and biliary leakage. *HPB* 2016; **18**:348–353.
147. Hirokawa F, Hayashi M, Miyamoto Y, Asakuma M, Shimizu T, Komeda K, et al. Evaluation of postoperative antibiotic prophylaxis after liver resection: a randomized controlled trial. *Am J Surg* 2013; **206**:8–15.
148. Zhou YM, Chen ZY, Li XD, Xu DH, Su X, Li B. Preoperative antibiotic prophylaxis does not reduce the risk of postoperative infectious complications in patients undergoing elective hepatectomy. *Dig Dis Sci* 2016; **61**:1707–1713.
149. Ren W, Wang X, Zhang A, Li C, Chen G, Ge X, et al. Selective bowel decontamination improves the survival of 90% hepatectomy in rats. *J Surg Res* 2015; **195**:454–464.
150. Gurusamy KS, Nagendran M, Davidson BR. Methods of preventing bacterial sepsis and wound complications after liver transplantation. Cochrane Database Syst Rev 2014:CD006660.
151. Verbeke L, Farre R, Trebicka J, Komuta M, Roskams T, Klein S, et al. Obeticholic acid, a farnesoid X receptor agonist, improves portal hypertension by two distinct pathways in cirrhotic rats. *Hepatology* 2014; **59**:2286–2298.
152. Hollman DA, Milona A, van Erpecum KJ, van Mil SW. Anti-inflammatory and metabolic actions of FXR: insights into molecular mechanisms. *Biochim Biophys Acta* 2012; **1821**:1443–1452.
153. Schaap FG, Trauner M, Jansen PL. Bile acid receptors as targets for drug development. *Nat Rev Gastroenterol Hepatol* 2014; **11**:55–67.
154. Neuschwander-Tetri BA, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, Abdelmalek MF, et al. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. *Lancet* 2015; **385**:956–965.
155. Keitel V, Donner M, Winandy S, Kubitz R, Haussinger D. Expression and function of the bile acid receptor TGR5 in Kupffer cells. *Biochem Biophys Res Commun* 2008; **372**:78–84.
156. Keitel V, Haussinger D. TGR5 in the biliary tree. *Dig Dis* 2011; **29**:45–47.
157. Wagner M, Halilbasic E, Marschall HU, Zollner G, Fickert P, Langner C, et al. CAR and PXR agonists stimulate hepatic bile acid and bilirubin detoxification and elimination pathways in mice. *Hepatology* 2005; **42**:420–430.
158. Tschuor C, Kachaylo E, Limani P, Raptis DA, Linecker M, Tian Y, et al. Constitutive androstane receptor (Car)-driven regeneration protects liver from failure following tissue loss. *J Hepatol* 2016; **65**:66–74.
159. van Dijk R, Kremer AE, Smit W, van den Elzen B, van Gulik T, Gouma D, et al. Characterization and treatment of persistent hepatocellular secretory failure. *Liver Int* 2015; **35**:1478–1488.
160. Hayashi H, Sakai K, Baba H, Sakai T. Thrombospondin-1 is a novel negative regulator of liver regeneration after partial hepatectomy through transforming growth factor-beta1 activation in mice. *Hepatology* 2012; **55**:1562–1573.
161. Kuroki H, Hayashi H, Nakagawa S, Sakamoto K, Higashi T, Nitta H, et al. Effect of LSKL peptide on thrombospondin 1-mediated transforming growth factor beta signal activation and liver regeneration after hepatectomy in an experimental model. *Br J Surg* 2015; **102**:813–825.

162. Stutchfield BM, Antoine DJ, Mackinnon AC, Gow DJ, Bain CC, Hawley CA, et al. CSF1 Restores Innate Immunity After Liver Injury in Mice and Serum Levels Indicate Outcomes of Patients with Acute Liver Failure. *Gastroenterology* 2015; **149**:1896–1909 e1814.
163. Forbes SJ, Gupta S, Dhawan A. Cell therapy for liver disease: from liver transplantation to cell factory. *J Hepatol* 2015;**62**: S157–S169.
164. Duncan AW, Dorrell C, Grompe M. Stem cells and liver regeneration. *Gastroenterology* 2009; **137**:466–481.
165. Huch M, Dorrell C, Boj SF, van Es JH, Li VS, van de Wetering M, et al. In vitro expansion of single Lgr5+ liver stem cells induced by Wnt-driven regeneration. *Nature* 2013; **494**:247–250.
166. Huang P, Zhang L, Gao Y, He Z, Yao D, Wu Z, et al. Direct reprogramming of human fibroblasts to functional and expandable hepatocytes. *Cell Stem Cell* 2014; **14**:370–384.
167. Takebe T, Sekine K, Enomura M, Koike H, Kimura M, Ogaeri T, et al. Vascularized and functional human liver from an iPSC-derived organ bud transplant. *Nature* 2013; **499**:481–484.
168. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006; **126**:663–676.
169. Kimura F, Shimizu H, Yoshidome H, Ohtsuka M, Kato A, Yoshitomi H, et al. Circulating cytokines, chemokines, and stress hormones are increased inpatients with organ dysfunction following liver resection. *J Surg Res* 2006; **133**:102–112.
170. van de Kerkhove MP, de Jong KP, Rijken AM, de Pont AC, van Gulik TM. MARS treatment in posthepatectomy liver failure. *Liver Int* 2003; **23**:44–51.
171. van Wenum M, Chamuleau RA, van Gulik TM, Siliakus A, Seppen J, Hoekstra R. Bioartificial livers in vitro and in vivo: tailoring biocomponents to the expanding variety of applications. *Expert Opin Biol Ther* 2014; **14**:1745–1760.
172. Lee KC, Baker LA, Stanzani G, Alibhai H, Chang YM, Jimenez Palacios C, et al. Extracorporeal liver assist device to exchange albumin and remove endotoxin in acute liver failure: results of a pivotal preclinical study. *J Hepatol* 2015; **63**:634–642.
173. Larsen FS, Schmidt LE, Bernsmeier C, Rasmussen A, Isoniemi H, Patel VC, et al. High-volume plasma exchange in patients with acute liver failure: an open randomised controlled trial. *J Hepatol* 2016; **64**:69–78.
174. Rifai K, Ernst T, Kretschmer U, Bahr MJ, Schneider A, Hafer C, et al. Prometheus–a new extracorporeal system for the treatment of liver failure. *J Hepatol* 2003; **39**:984–990.
175. Otsuka Y, Duffy JP, Saab S, Farmer DG, Ghobrial RM, Hiatt JR, et al. Postresection hepatic failure: successful treatment with liver transplantation. *Liver transpl* 2007; **13**:672–679.
176. van den Broek MA, van Dam RM, Malago M, Dejong CH, van Breukelen GJ, Olde Damink SW. Feasibility of randomized controlled trials in liver surgery using surgery-related mortality or morbidity as endpoint. *Br J Surg* 2009; **96**:1005–1014.
177. van den Broek MA, van Dam RM, van Breukelen GJ, Bemelmans MH, Oussoultzoglou E, Pessaux P, et al. Development of a composite endpoint for randomized controlled trials in liver surgery. *Br J Surg* 2011; **98**:1138–1145.
178. Arroyo V, Moreau R, Jalan R, Gines P. Study E-CCC. Acute-on-chronic liver failure: a new syndrome that will re-classify cirrhosis. *J Hepatol* 2015; **62**: S131–S1

Chapter 3

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

Kim M.C. van Mierlo, Junfang Zhao, Jos Kleijnen, Sander S. Rensen, Frank G. Schaap, Cornelis H.C. Dejong, Steven W.M. Olde Damink

***Surgical Oncology*. 2016 Sep;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). We aimed 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 [0.15, 6.89] (p=0.98) for liver failure, an OR of 1.21 [0.23, 6.35] (p=0.82) for overall mortality, and an OR of 3.52 [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.

Conclusion. 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 neoadjuvant 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 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 study protocol can be found in Appendix 1 (online: doi: 10.1016/j.suronc.2016.05.030). 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 (<18 years old). 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.

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 resection was defined as resection of 3 or more 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

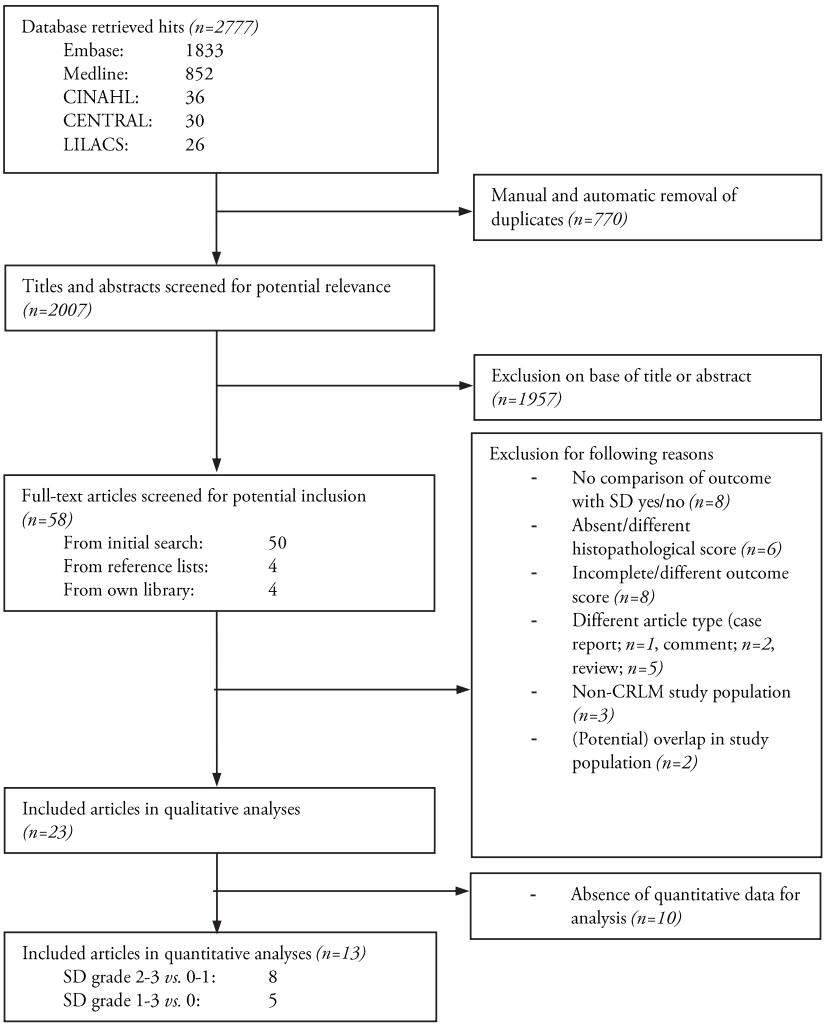
Meta-analyses of two or more studies per outcome were performed in Review Manager 5.3 and depicted in forest plots. The principal effect measures consisted of a pooled odds ratio (OR) with 95% confidence intervals (95% CI) calculated by the Mantel-Haenszel test for dichotomous outcomes, which was chosen 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 between 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 of 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 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

Figure 1. Selection process of included studies



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

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 *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. ˣ 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.

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 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 two were retrospective cohort studies which enrolled at least 50 patients.[22,56] 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]

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 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

Table 1. Summary of study characteristics and risk of bias assessment

Name	Year	Country	n	Comparison	Study design	Quant/ Qual	QUIPS score	Main outcome
Aloysius[42]	2007	UK	50	3 *vs.* 2 *vs.* 1 *vs.* 0	RCohS	Qual	High	Folfox-4 therapy is associated with SD and hepatic steatosis
Brouquet[43]	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[44]	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[45]	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[46]	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[47]	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 ≥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

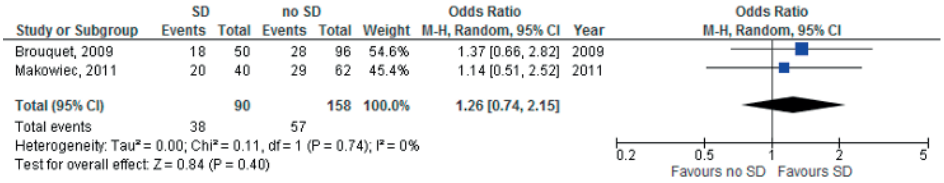
Table 1. Summary of study characteristics and risk of bias assessment (continued)

Name	Year	Country	n	Comparison	Study design	Quant/ Qual	QUIPS score	Main outcome
*Narita[32]	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
*Narita[48]	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[49]	2013	France	50	1-3 *vs.* 0	RCohS	Quan	Low	Ctx of CRLM induces SD related to oxaliplatin, without impact on postoperative mortality
×Pessaux[50]	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
×Pessaux[51]	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[52]	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	Nether-lands	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 CALI
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 *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. × 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.

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 2A. Postoperative overall morbidity

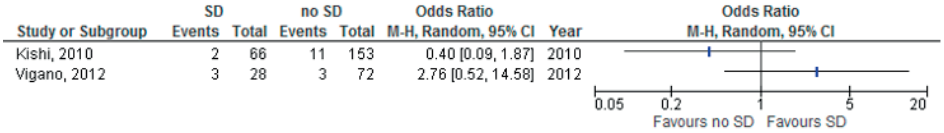


SD; sinusoidal dilatation, M-H; Mantel-Haenszel, Random; random effects model, 95% CI; 95% confidence interval

Postoperative liver failure

The effect of SD on postoperative liver failure (PLF) could be tested in two studies (Figure 2B). In the study of Kishi et al., liver failure was defined as peak total bilirubin value > 120 µmol/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 serum bilirubin >50 µmol/L and prothrombin time <50% 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 2B. Postoperative liver failure



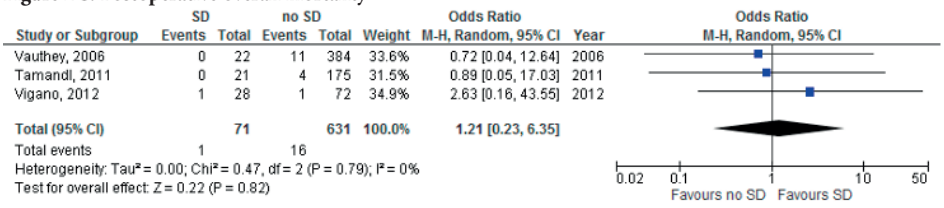
SD; sinusoidal dilatation, M-H; Mantel-Haenszel, Random; random effects model, 95% CI; 95% confidence interval

Postoperative mortality

Three studies were available for examining the influence of SD on postoperative mortality (Figure 2C), with a total of 17 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*=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

SD.[47] 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.

Figure 2C. Postoperative overall mortality



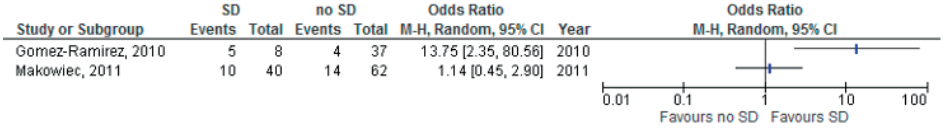
SD; sinusoidal dilatation, M-H; Mantel-Haenszel, Random; random effects model, 95% CI; 95% confidence interval

Secondary outcome

Liver-related morbidity

Two studies were available for estimating the influence of SD on postoperative liver-related morbidity (Figure 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 2D. Postoperative liver-related morbidity



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

Subgroup 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 3. 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 3A). 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 µmol/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 3B). 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 3C). For major resections only, forest plots were constructed for postoperative overall morbidity and liver failure (Appendix 4). Two studies that included a total of 137 patients were available for a meta-analysis on postoperative morbidity (Appendix 4A).[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 4B). Whereas Nakano et al. defined postoperative liver failure as a serum bilirubin >50 µmol/L and a prothrombin time <50% on or after POD5,[20] Narita et al. defined this as a serum bilirubin >50 µmol/L and a prothrombin time <50% on POD5 and/or a postoperative serum bilirubin >120 µmol/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 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 5.

GRADE assessment

Study findings were assessed with the modified GRADE checklist[42,58] and are shown in the Summary of Findings Table (Table 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 considered 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 6. 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 7 and 8.

Table 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

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[1]
Morbidity Follow-up: 30 to 90 days or in-hospital	248	2	OR 1.26 (0.74 to 2.15)	Very low[2,3,6]
Liver failure Follow-up: 30 to 90 days or in-hospital	319	2	Omitted due to significant heterogeneity	Very low[2,3,4,5,6]
Mortality Follow-up: 30 to 90 days or in-hospital	702	3	OR 1.21 (0.23 to 6.35)	Very low[4,6]
Liver-related morbidity Follow-up: 30 to 90 days or in-hospital	147	2	Omitted due to significant heterogeneity	Very low[2,3,4,5]

[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), whereas phase 1 explanatory studies start with a moderate score (three points).[30] Since all outcomes consisted for ≥50% of phase 1 studies, the starting score for all outcomes was set on three points.
[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 95% confidence 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 $I^2 \geq 65\%$ 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

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, 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. Considering these findings, no solid conclusions can be drawn, and this study cannot provide clinical advice on the topic. 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 not include 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 the 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 preoperative 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. Regarding 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 after liver surgery in order to ensure clear and consistent clinically relevant data, and to allow comparison 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 outcomes in surgical oncology.

ACKNOWLEDGMENTS

The authors would like to thank information specialist Ms. Janine Ross for performing the extensive search used in this study. Furthermore, special thanks go to Dr. Jill Hayden for her advice on application of the QUIPS and GRADE framework for prognosis studies.

REFERENCES

1. L. Rubbia-Brandt, V. Audard, P. Sartoretti, A.D. Roth, C. Brezault, M. Le Charpentier, B. Dousset, P. Morel, O. Soubrane, S. Chaussade, G. Mentha, B. Terris. Severe hepatic sinusoidal obstruction associated with oxaliplatin-based chemotherapy in patients with metastatic colorectal cancer. *Ann Oncol* 2004; **15**:460-466.
2. L. Rubbia-Brandt, G.Y. Lauwers, H. Wang, P.E. Majno, K. Tanabe, A.X. Zhu, C. Brezault, O. Soubrane, E.K. Abdalla, J.-N.N. Vauthey, G. Mentha, B. Terris. Sinusoidal obstruction syndrome and nodular regenerative hyperplasia are frequent oxaliplatin-associated liver lesions and partially prevented by bevacizumab in patients with hepatic colorectal metastasis. *Histopathology* 2010; **56**:430-439.
3. S.M. Robinson, C.H. Wilson, A.D. Burt, D.M. Manas, S.A. White. Chemotherapy associated liver injury in patients with colorectal liver metastases: a systematic review and meta-analysis. *Ann Surg Oncol* 2012; **19**:4287-4299.
4. V.W. Lam, C. Spiro, J.M. Laurence, E. Johnston, M.J. Hollands, H.C. Pleass, A.J. Richardson. A systematic review of clinical response and survival outcomes of downsizing systemic chemotherapy and rescue liver surgery in patients with initially unresectable colorectal liver metastases. *Ann Surg Oncol* 2012; **19**:1292-1301.
5. Y. Seium, R. Stupp, T. Ruhstaller, P. Gervaz, G. Mentha, M. Philippe, A. Allal, C. Trembleau, J. Bauer, R. Morant, A.D. Roth. Oxaliplatin combined with irinotecan and 5-fluorouracil/leucovorin (OCFL) in metastatic colorectal cancer: a phase I-II study. *Ann Oncol* 2005; **16**:762-766.
6. R. Adam, V. Delvart, G. Pascal, A. Valeanu, D. Castaing, D. Azoulay, S. Giacchetti, B. Paule, F. Kunstlinger, O. Ghemard, F. Levi, H. Bismuth. Rescue surgery for unresectable colorectal liver metastases downstaged by chemotherapy: a model to predict long-term survival. *Ann Surg* 2004; **240**:644-657.
7. J. Agostini, S. Benoist, M. Seman, C. Julie, S. Imbeaud, F. Letourneur, N. Cagnard, P. Rougier, A. Brouquet, J. Zucman-Rossi, P. Laurent-Puig. Identification of molecular pathways involved in oxaliplatin-associated sinusoidal dilatation. *J Hepatol* 2012; **56**:869-876.
8. K. Nakamura, E. Hatano, A. Miyagawa-Hayashino, M. Okuno, Y. Koyama, M. Narita, S. Seo, K. Taura, S. Uemoto. Soluble thrombomodulin attenuates sinusoidal obstruction syndrome in rat through suppression of high mobility group box 1. *Liver Int* 2014; **34**:1473-1487.
9. S.M. Robinson, J. Mann, A. Vasilaki, J. Mathers, A.D. Burt, F. Oakley, S.A. White, D.A. Mann. Pathogenesis of FOLFOX induced sinusoidal obstruction syndrome in a murine chemotherapy model. *J Hepato*l 2013; **59**:318-326.
10. X. Wang, G.C. Kanel, L.D. DeLeve. Support of sinusoidal endothelial cell glutathione prevents hepatic veno-occlusive disease in the rat. *Hepatology* 2000; **31**:428-434.
11. L.D. DeLeve, Y. Ito, N.W. Bethea, M.K. McCuskey, X. Wang, R.S. McCuskey, Embolization by sinusoidal lining cells obstructs the microcirculation in rat sinusoidal obstruction syndrome. *Am J Physiol Gastrointest Liver Physiol* 2003; **284**:1045-1052.
12. T. Ezzat, M.A. van den Broek, N. Davies, C.H. Dejong, A. Bast, M. Malago, D.K. Dhar, S.W. Olde Damink. The flavonoid monoHER prevents monocrotaline-induced hepatic sinusoidal injury in rats. *J Surg Oncol* 2012; **106**:72-78.
13. C.Q. Fan, J.M. Crawford. Sinusoidal obstruction syndrome (hepatic venoocclusive disease). *J Clin Exp Hepatol* 2014; **4**:332-346.
14. L.D. DeLeve, R.S. McCuskey, X. Wang, L. Hu, M.K. McCuskey, R.B. Epstein, G.C. Kanel. Characterization of a reproducible rat model of hepatic venoocclusive disease. *Hepatology* 1999; **29**:1779-1791.

15. G. Mentha, S. Terraz, P. Morel, A. Andres, E. Giostra, A. Roth, L. Rubbia-Brandt, P. Majno, Dangerous halo after neoadjuvant chemotherapy and two-step hepatectomy for colorectal liver metastases. *Br J Surg* 2009; **96**:95-103.
16. M.J. Overman, D.M. Maru, C. Charnsangavej, E.M. Loyer, H. Wang, P. Pathak, C. Eng, P.M. Hoff, J.N. Vauthey, R.A. Wolff, S. Kopetz. Oxaliplatin-mediated increase in spleen size as a biomarker for the development of hepatic sinusoidal injury. *J Clin Oncol* 2010; **28**:2549-2555.
17. R. Arotcarena, V. Cales, P. Berthelemy, Y. Parent, M. Malet, F. Etcharry, S. Ferrari, A. Pariente. Severe sinusoidal lesions: a serious and overlooked complication of oxaliplatin-containing chemotherapy? *Gastroenterol Clin Biol* 2006; **30**:1313-1316.
18. J.H. Slade, M.L. Alattar, D.R. Fogelman, M.J. Overman, A. Agarwal, D.M. Maru, R.L. Coulson, C. Charnsangavej, J.N. Vauthey, R.A. Wolff, S. Kopetz. Portal hypertension associated with oxaliplatin administration: clinical manifestations of hepatic sinusoidal injury. *Clin Colorectal Cancer* 2009; **8**:225-230.
19. K. Imai, Y. Emi, K.I. Iyama, T. Beppu, Y. Ogata, Y. Kakeji, H. Samura, E. Oki, Y. Akagi, Y. Maehara, H. Baba, Kyusyu Study Group of Clinical Cancer ancillary. Splenic volume may be a useful indicator of the protective effect of bevacizumab against oxaliplatin-induced hepatic sinusoidal obstruction syndrome. *Eur J Surg Oncol* 2014; **40**:559-566.
20. H. Nakano, E. Oussoultzoglou, E. Rosso, S. Casnedi, M.P. Chenard-Neu, P. Dufour, P. Bachellier, D. Jaeck. Sinusoidal injury increases morbidity after major hepatectomy in patients with colorectal liver metastases receiving preoperative chemotherapy. *Ann Surg* 2008; **247**:118-124.
21. D. Tamandl, M. Klinger, S. Eipeldauer, B. Herberger, K. Kaczirek, B. Gruenberger, T. Gruenberger. Sinusoidal obstruction syndrome impairs long-term outcome of colorectal liver metastases treated with resection after neoadjuvant chemotherapy. *Ann Surg Oncol* 2011; **18**:421-430.
22. J. Gomez-Ramirez, E. Martin-Perez, C.G. Amat, I.G. Sanz, E. Bermejo, A. Rodriguez, E. Larranaga. Influence of pre-surgical chemotherapy on liver parenchyma and post-surgical outcome of patients subjected to hepatectomy due to colorectal carcinoma metastases. *Cir Esp* 2010; **88**:404-412.
23. T. Takamoto, T. Hashimoto, K. Sano, Y. Maruyama, K. Inoue, S. Ogata, T. Takemura, N. Kokudo, M. Makuuchi. Recovery of liver function after the cessation of preoperative chemotherapy for colorectal liver metastasis. *Ann Surg Oncol* 2010; **17**:2747-2755.
24. S. Kandutsch, M. Klinger, S. Hacker, F. Wrba, B. Gruenberger, T. Gruenberger. Patterns of hepatotoxicity after chemotherapy for colorectal cancer liver metastases. *Eur J Surg Oncol* 2008; **34**:1231-1236.
25. H. Komori, T. Beppu, Y. Baba, K. Horino, C. Imsung, T. Masuda, H. Hayashi, H. Okabe, R. Ootao, M. Watanabe, H. Takamori, K. Iyama, H. Baba. Histological liver injury and surgical outcome after FOLFOX followed by a hepatectomy for colorectal liver metastases in Japanese patients. *Int J Clin Oncol* 2010; **15**:263-270.
26. F. Makowiec, S. Mohrle, H. Neeff, O. Drognitz, G. Illerhaus, O.G. Opitz, U.T. Hopt, A. zur Hausen. Chemotherapy, liver injury, and postoperative complications in colorectal liver metastases. *J Gastrointest Surg* 2011; **15**:153-164.
27. T.M. Pawlik, K. Olino, A.L. Gleisner, M. Torbenson, R. Schulick, M.A. Choti. Preoperative chemotherapy for colorectal liver metastases: impact on hepatic histology and postoperative outcome. *J Gastrointest Surg* 2007; **11**:860-868.
28. A. Sahajpal, C.M. Vollmer, E. Dixon, E.K. Chan, A. Wei, M.S. Cattral, B.R. Taylor, D.R. Grant, P.D. Greig, S. Gallinger. Chemotherapy for colorectal cancer prior to liver resection for colorectal cancer hepatic metastases does not adversely affect peri-operative outcomes. *J Surg Oncol* 2007; **95**:22-27.

29. D. Takeda, H. Nitta, T. Takahara, Y. Hasegawa, N. Itou, G. Wakabayashi. Effect of preoperative chemotherapy on postoperative liver regeneration following hepatic resection as estimated by liver volume. *World J Surg Oncol* 2013; **11**:65.
30. J.A. Hayden, M.E. Tougas, R. Riley, R. Iles, T. Pincus. Individual recovery expectations and prognosis of outcomes in non-specific low back pain: prognostic factor exemplar review (Protocol). *Cochrane Database Syst Rev* 2014; **9**.
31. D. Moher, A. Liberati, J. Tetzlaff, D.G. Altman, P. Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med* 2009; **151**:264-269.
32. D.F. Stroup, J.A. Berlin, S.C. Morton, I. Olkin, G.D. Williamson, D. Rennie, D. Moher, B.J. Becker, T.A. Sipe, S.B. Thacker, M. Grp. Meta-analysis of observational studies in epidemiology - a proposal for reporting. *JAMA* 2000; **283**:2008-2012.
33. M. Narita, E. Oussoultzoglou, M.P. Chenard, E. Rosso, S. Casnedi, P. Pessaux, P. Bachellier, D. Jaeck. Sinusoidal obstruction syndrome compromises liver regeneration in patients undergoing two-stage hepatectomy with portal vein embolization. *Surg Today* 2011; **41**:7-17.
34. CADTH, Canadian Agency for Drugs and Technologies in Health. CADTH Peer Review Checklist for Search Strategies 2013, 3.
35. D. Dindo, N. Demartines, P.A. Clavien. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. *Ann Surg* 2004; **240**:205-213.
36. S. Balzan, J. Belghiti, O. Farges, S. Ogata, A. Sauvanet, D. Delefosse, F. Durand. The "50-50 criteria" on postoperative day 5: an accurate predictor of liver failure and death after hepatectomy. *Ann Surg* 2005; **242**:824-828.
37. J.T. Mullen, D. Ribero, S.K. Reddy, M. Donadon, D. Zorzi, S. Gautam, E.K. Abdalla, S.A. Curley, L. Capussotti, B.M. Clary, J.N. Vauthey. Hepatic insufficiency and mortality in 1,059 noncirrhotic patients undergoing major hepatectomy. *J Am Coll Surg* 2007; **204**:854-862.
38. N.N. Rahbari, O.J. Garden, R. Padbury, M. Brooke-Smith, M. Crawford, R. Adam, M. Koch, M. Makuuchi, R.P. Dematteo, C. Christophi, S. Banting, V. Usatoff, M. Nagino, G. Maddern, T.J. Hugh, J.N. Vauthey, P. Greig, M. Rees, Y. Yokoyama, S.T. Fan, Y. Nimura, J. Figueras, L. Capussotti, M.W. Buchler, J. Weitz. Posthepatectomy liver failure: a definition and grading by the international study group of liver surgery (ISGLS). *Surgery* 2011; **149**:713-724.
39. M.A. van den Broek, R.M. van Dam, G.J. van Breukelen, M.H. Bemelmans, E. Oussoultzoglou, P. Pessaux, C.H. Dejong, N. Freemantle, S.W. Olde Damink. Development of a composite endpoint for randomized controlled trials in liver surgery. *Br J Surg* 2011; **98**:1138-1145.
40. J.A. Hayden, P. Cote, C. Bombardier. Evaluation of the quality of prognosis studies in systematic reviews. *Ann Intern Med* 2006; **144**:427-437.
41. J.A. Hayden, D.A. van der Windt, J.L. Cartwright, P. Cote, C. Bombardier. Assessing bias in studies of prognostic factors. *Ann Intern Med* 2013; **158**:280-286.
42. P. Davis, J. Hayden, J. Springer, J. Bailey, M. Molinari, P. Johnson. Prognostic factors for morbidity and mortality in elderly patients undergoing acute gastrointestinal surgery: a systematic review. *Can J Surg* 2014; **57**:44-52.
43. M.M. Aloysius, A.M. Zaitoun, I.J. Beckingham, K.R. Neal, G.P. Aithal, E.M. Bessell, D.N. Lobo. The pathological response to neoadjuvant chemotherapy with FOLFOX-4 for colorectal liver metastases: a comparative study. *Virchows Arch* 2007; **451**:943-948.
44. A. Brouquet, S. Benoist, C. Julie, C. Penna, A. Beauchet, P. Rougier, B. Nordlinger. Risk factors for chemotherapy-associated liver injuries: a multivariate analysis of a group of 146 patients with colorectal metastases. *Surgery* 2009; **145**:362-371.

45. C. Hubert, C. Fervaille, C. Sempoux, Y. Horsmans, Y. Humblet, J.P. Machiels, F. Zech, A. Ceratti, J.F. Gigot. Prevalence and clinical relevance of pathological hepatic changes occurring after neoadjuvant chemotherapy for colorectal liver metastases. *Surgery* 2010; **147**:185-194.
46. M. Karoui, C. Penna, M. Amin-Hashem, E. Mitry, S. Benoist, B. Franc, P. Rougier, B. Nordlinger. Influence of preoperative chemotherapy on the risk of major hepatectomy for colorectal liver metastases. *Ann Surg* 2006; **243**:1-7.
47. Y. Kishi, D. Zorzi, C.M. Contreras, D.M. Maru, S. Kopetz, D. Ribero, M. Motta, N. Ravarino, M. Risio, S.A. Curley, E.K. Abdalla, L. Capussotti, J.N. Vauthey. Extended preoperative chemotherapy does not improve pathologic response and increases postoperative liver insufficiency after hepatic resection for colorectal liver metastases. *Ann Surg Oncol* 2010; **17**:2870-2876.
48. K. Miura, H. Nakano, J. Sakurai, S. Kobayashi, S. Koizumi, T. Arai, T. Shimamura, R. Makizumi, K. Yamada, N. Miyajima, T. Otsubo, J. Koike. Splenomegaly in FOLFOX-naive stage IV or recurrent colorectal cancer patients due to chemotherapy-associated hepatotoxicity can be predicted by the aspartate aminotransferase to platelet ratio before chemotherapy. *Int J Clin Oncol* 2011; **16**:257-263.
49. M. Narita, E. Oussoultzoglou, P. Fuchshuber, P. Pessaux, M.P. Chenard, E. Rosso, C. Nobili, D. Jaeck, P. Bachellier. What is a safe future liver remnant size in patients undergoing major hepatectomy for colorectal liver metastases and treated by intensive preoperative chemotherapy? *Ann Surg Oncol* 2012; **19**:2526-2538.
50. E. Nguyen-Khac, C. Lobry, D. Chatelain, D. Fuks, J.P. Joly, M. Brevet, B. Tramier, C. Mouly, V. Hautefeuille, B. Chauffert, J.M. Regimbeau. A reappraisal of chemotherapy-induced liver injury in colorectal liver metastases before the era of antiangiogenics. Int J Hepatol 2013.
51. P. Pessaux, E. Marzano, S. Casnedi, P. Bachellier, D. Jaeck, M.P. Chenard. Histological and immediate postoperative outcome after preoperative cetuximab: case-matched control study. *World J Surg* 2010; **34**:2765-2772.
52. P. Pessaux, F. Panaro, S. Casnedi, I. Zeca, E. Marzano, P. Bachellier, D. Jaeck, M.P. Chenard. Targeted molecular therapies (cetuximab and bevacizumab) do not induce additional hepatotoxicity: preliminary results of a case-control study. *Eur J Surg Oncol* 2010; **36**:575-582.
53. O. Soubrane, A. Brouquet, S. Zalinski, B. Terris, C. Brezault, V. Mallet, F. Goldwasser, O. Scatton. Predicting high grade lesions of sinusoidal obstruction syndrome related to oxaliplatin-based chemotherapy for colorectal liver metastases: correlation with post-hepatectomy outcome. *Ann Surg* 2010; **251**:454-460.
54. A.E. van der Pool, H.A. Marsman, J. Verheij, F.J. Ten Kate, A.M. Eggermont, J.N. Ijzermans, C. Verhoef. Effect of bevacizumab added preoperatively to oxaliplatin on liver injury and complications after resection of colorectal liver metastases. *J Surg Oncol* 2012; **106**:892-897.
55. J.N. Vauthey, T.M. Pawlik, D. Ribero, T.T. Wu, D. Zorzi, P.M. Hoff, H.Q. Xiong, C. Eng, G.Y. Lauwers, M. Mino-Kenudson, M. Risio, A. Muratore, L. Capussotti, S.A. Curley, E.K. Abdalla. Chemotherapy regimen predicts steatohepatitis and an increase in 90-day mortality after surgery for hepatic colorectal metastases. *J Clin Oncol* 2006; **24**:2065-2072.
56. L. Vigano, N. Ravarino, A. Ferrero, M. Motta, B. Torchio, L. Capussotti. Prospective evaluation of accuracy of liver biopsy findings in the identification of chemotherapy-associated liver injuries. *Arch Surg* 2012; **147**:1085-1091.
57. P.S. Wolf, J.O. Park, F. Bao, P.J. Allen, R.P. DeMatteo, Y. Fong, W.R. Jarnagin, T.P. Kingham, M. Gonen, N. Kemeny, J. Shia, M.I. D'Angelica. Preoperative chemotherapy and the risk of hepatotoxicity and morbidity after liver resection for metastatic colorectal cancer: a single institution experience. *J Am Coll Surg* 2013; **216**:41-49.

58. A. Iorio, F.A. Spencer, M. Falavigna, C. Alba, E. Lang, B. Burnand, T. McGinn, J. Hayden, K. Williams, B. Shea, R. Wolff, T. Kujpers, P. Perel, P.O. Vandvik, P. Glasziou, H. Schunemann, G. Guyatt. Use of GRADE for assessment of evidence about prognosis: rating confidence in estimates of event rates in broad categories of patients. *BMJ* 2015; **350**:870.
59. A. Huguet, J.A. Hayden, J. Stinson, P.J. McGrath, C.T. Chambers, M.E. Tougas, L. Wozney. Judging the quality of evidence in reviews of prognostic factor research: adapting the GRADE framework. *Syst Rev* 2013; **2**:71.
60. S. Stremitzer, J. Stift, J. Singh, P. Starlinger, B. Gruenberger, D. Tamandl, T. Gruenberger. Histological response, pattern of tumor destruction and clinical outcome after neoadjuvant chemotherapy including bevacizumab or cetuximab in patients undergoing liver resection for colorectal liver metastases. *Eur J Surg Oncol* 2015; **41**:868-874.
61. D. Ribero, H. Wang, M. Donadon, D. Zorzi, M.B. Thomas, C. Eng, D.Z. Chang, S.A. Curley, E.K. Abdalla, L.M. Ellis, J.N. Vauthey. Bevacizumab improves pathologic response and protects against hepatic injury in patients treated with oxaliplatin-based chemotherapy for colorectal liver metastases. *Cancer* 2007; **110**:2761-2767.
62. P. Economopoulou, A. Kotsakis, I. Kapiris, N. Kentepozidis. Cancer therapy and cardiovascular risk: focus on bevacizumab. *Cancer Manag Res* 2015; **7**:133-143.
63. Z.V. Varga, P. Ferdinandy, L. Liaudet, P. Pacher. Drug-induced mitochondrial dysfunction and cardiotoxicity. *Am J Physiol Heart Circ Physiol* 2015; **309**:1453-1467.
64. B. Aussilhou, S. Dokmak, S. Faivre, V. Paradis, V. Vilgrain, J. Belghiti. Preoperative liver hypertrophy induced by portal flow occlusion before major hepatic resection for colorectal metastases can be impaired by bevacizumab. *Ann Surg Oncol* 2009; **16**:1553-1559.
65. B. Gruenberger, D. Tamandl, J. Schueller, W. Scheithauer, C. Zielinski, F. Herbst, T. Gruenberger. Bevacizumab, capecitabine, and oxaliplatin as neoadjuvant therapy for patients with potentially curable metastatic colorectal cancer. *J Clin Oncol* 2008; **26**:1830-1835.
66. C.H. Dejong. Vascular reconstruction combined with liver resection for malignant tumours. *Br J Surg* 2013; **100**:1776.
67. S.K. Reddy, T.M. Pawlik, D. Zorzi, A.L. Gleisner, D. Ribero, L. Assumpcao, A.S. Barbas, E.K. Abdalla, M.A. Choti, J.N. Vauthey, K.A. Ludwig, C.R. Mantyh, M.A. Morse, B.M. Clary. Simultaneous resections of colorectal cancer and synchronous liver metastases: a multi-institutional analysis. *Ann Surg Oncol* 2007; **14**:3481-3491.
68. K.P. van Lienden, J.W. van den Esschert, W. de Graaf, S. Bipat, J.S. Lameris, T.M. van Gulik, O.M. van Delden. Portal vein embolization before liver resection: a systematic review. *Cardiovasc Interv Radiol* 2013; **36**:25-34.
69. J.N. Vauthey, A. Chaoui, K.A. Do, M.M. Bilimoria, M.J. Fenstermacher, C. Charnsangavej, M. Hicks, G. Alsfasser, G. Lauwers, I.F. Hawkins, J. Caridi. Standardized measurement of the future liver remnant prior to extended liver resection: methodology and clinical associations. *Surgery* 2000; **127**:512-519.
70. R.D. Riley, P.C. Lambert, G. Abo-Zaid. Meta-analysis of individual participant data: rationale, conduct, and reporting. *BMJ* 2010; **340**.

SUPPLEMENTAL DATA

Appendix 1. Study protocol

The study protocol is available online via: doi: 10.1016/j.suronc.2016.05.030

Appendix 2. 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$)).ti,ab,ot. (26)
3 Stuart bras syndrome.ti,ab,ot. (0)
4 ((SOS or VOD or HVOD) adj5 (liver or livers or hepatic$ 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$)).ti,ab,ot. (887)
3 Stuart bras syndrome.ti,ab,ot. (3)
4 ((SOS or VOD or HVOD) adj5 (liver or livers or hepatic$ 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 trees40
#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

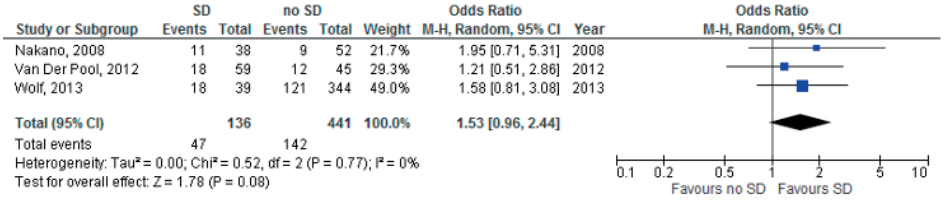
Literature in the Health Sciences in Latin America and the Caribbean (LILACS) (Internet): 2004-2015/06/03
Searched 9.6.15
Advanced search
Limited to 2004-2015
Limited to Humans only
Limited to: LILACS
(tw:((mh: c06.552.360 OR mh: c14.907.460 OR mh: hepatic veno-occlusive disease OR sinusoidal obstruction OR sinusoidal injury OR „stuart bras syndrome“ OR „Enfermedad Veno-Oclusiva Hepática“ OR „Hepatopatia 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 3. Sub analyses of postoperative short-term outcomes SD grade 1-3 vs. SD grade 0

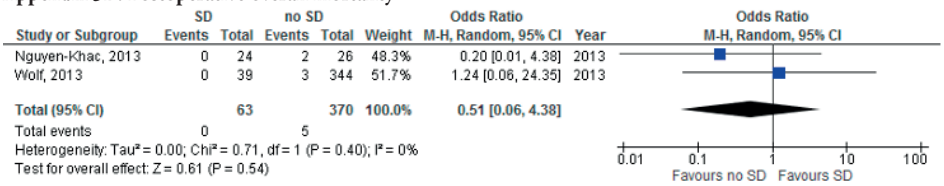
Meta-analyses on the influence of sinusoidal dilatation (SD) grade 1-3 versus SD grade 0 on the complication rate after partial hepatectomy for colorectal liver metastases

Appendix 3A. Postoperative overall morbidity



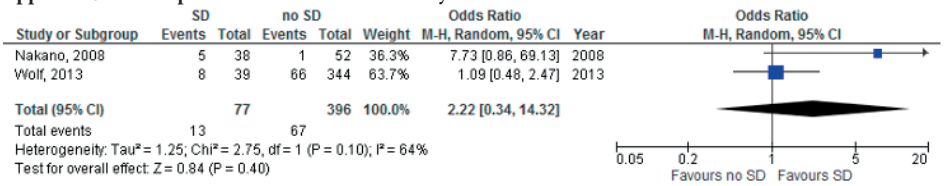
SD; sinusoidal dilatation, M-H; Mantel-Haenszel, Random; random effects model, 95% CI; 95% confidence interval

Appendix 3B. Postoperative overall mortality



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

Appendix 3C. Postoperative liver-related morbidity

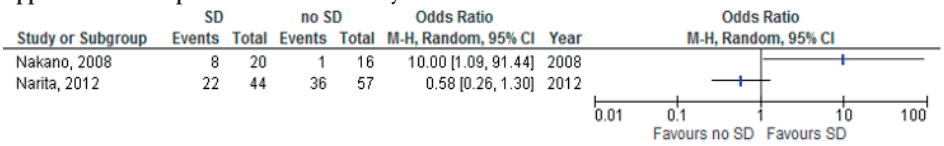


SD; sinusoidal dilatation, M-H; Mantel-Haenszel, Random; random effects model, 95% CI; 95% confidence interval

Appendix 4. Sub analyses of postoperative short-term outcomes SD grade 1-3 vs. SD grade 0 – major liver resections

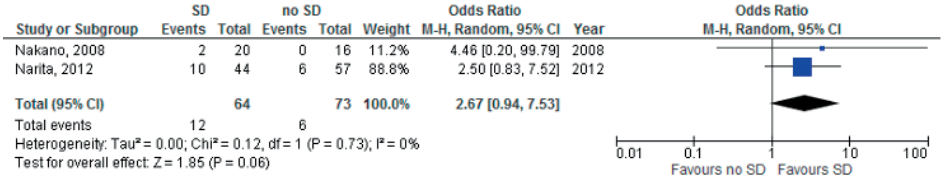
Meta-analyses on the influence of sinusoidal dilatation (SD) grade 1-3 versus SD grade 0 on the complication rate after major hepatectomy for colorectal liver metastases

Appendix 4A. Postoperative overall morbidity



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

Appendix 4B. Postoperative liver failure



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

Appendix 5. 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

	Aloysius[43]	Brou-quet[44]	Gómez[22]	Hubert[46]	Imai[19]	Kan-dutsch[24]	Karoui[46]	Kishi[47]
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	high	low	low
2. Study Attrition	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
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
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
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
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
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
Overall assessment	high	high	low	low	high	high	low	low

PF; prognostic factor, ü; yes, ü¯; partial, û; no, ?; unsure, mod; moderate, n/a; not applicable

	Komori[25]	Mako-wiec[26]	Mentha[15]	Miura[48]	Nakano[20]	Narita[49]	Nguyen[50]	Pessaux[52]
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	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
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
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	high	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	mod	mod	high	mod	mod	low	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	high	mod	mod	low	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	high	low	high	high	mod	low	low	high
Overall assessment	high	low	high	high	mod	low	low	high

PF; prognostic factor, ü; yes, ü-; partial, û; no, ?; unsure, mod; moderate, n/a; not applicable

	Soubra-ne[53]	Taka-moto[23]	Tamandl[21]	Van der Pool[54]	Vauthey[55]	Vigano[56]	Wolf[57]
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
2. Study Attrition	n/a	n/a	n/a	n/a	n/a	n/a	n/a
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
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
PF Measurement Summary	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	low	mod	low	mod	mod	low	mod
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	high	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	low	mod	low	low	low	low	mod
Overall assessment	low	mod	high	low	low	low	mod

PF; prognostic factor, ü; yes, ü-; partial, û; no, ?; unsure, mod; moderate, n/a; not applicable

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

Appendix 6. 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 1.2 (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 7. 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 Follow-up: 30 to 90 days or in-hospital	577	3	OR 1.53 (0.96 to 2.44)	Very low[2,3,6]
Liver failure Follow-up: 30 to 90 days or in-hospital	0	0	Not estimable	n/a
Mortality Follow-up: 30 to 90 days or in-hospital	433	2	OR 0.51 (0.06 to 4.38)	Very low[4,6]
Liver-related morbidity Follow-up: 30 to 90 days or in-hospital	473	2	OR 2.22 (0.34 to 14.32)	Very low[2,3,4,6]

[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 $I^2 \geq 65\%$ 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.

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

Appendix 8. 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**											
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)	OR 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)	OR 0.51 (0.06 to 4.38)	1	✓	×	✓	×	✓	n/a	n/a	+
Liver-related morbidity	473 (2)	OR 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 4

The influence of chemotherapy-associated liver injury on outcome after partial hepatectomy for colorectal liver metastases: a systematic review and individual participant data analysis

Kim M.C. van Mierlo, Junfang Zhao, Joaquín Gómez-Ramírez J, Haeryoung Kim, Charles H.C. Pilgrim, Patrick Pessaux, Sander S. Rensen, Eric P. van der Stok, Frank G. Schaap, Olivier Soubrane, Takeshi Takamoto, Luca Viganò, Bjorn Winkens, Cornelis H.C. Dejong, Steven W.M. Olde Damink; Chemotherapy-Associated Liver Injury (CALI) consortium

British Journal of Surgery. 2017 Jul;104(8):990-1002

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 (i.e., 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 01.01.2004 and 31.12.2013 with keywords: "chemotherapy", "liver resection", "outcome", and "colorectal metastases" to identify potential collaborating centres. Uni- and multivariable analyses were performed using binary logistic regression models and depicted in odds ratio (OR) with 95% confidence interval (CI).

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). Moreover, 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).

Conclusion. 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 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 irresectable, neoadjuvant systemic chemotherapy can prolong survival, and allow potential 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 irresectable 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 an increased occurrence of nodular regenerative hyperplasia (NRH).[11] Coadministration of bevacizumab with oxaliplatin, however, has been reported to be associated with a decrease in both 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, since 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 (i.e., postoperative morbidity, mortality),[9,13,15-18] others could not reproduce this.[19-23] 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

Inclusion criteria for this study

An extensive protocol, written before the start of this study, can be found in Appendix 1 (online: doi: 10.1002/bjs.10572). The PRISMA and Moose guidelines were followed for conducting and reporting this review.[24,25] Studies meeting the following criteria were considered eligible for inclusion: (a) adult patients (>18 years), (b) who underwent liver resection for CRLM, (c) with description of postoperative short-term overall morbidity, liver surgery-specific complications, postoperative liver failure, or overall mortality (≤90 days or in-hospital) after liver resection and, (d) with pathological assessment of non-tumorous liver specimens for SD, steatosis, and/or steatohepatitis. Studies with patients that received preoperative hepatic arterial infusion of chemotherapy were excluded, as were case reports, comments, published abstracts only, editorials, and reviews.

Search strategy for identification of studies

Systematic searches were performed (JZ, KvM) in Medline (PubMed) and Embase for studies published between 01.01.2004 and 31.12.2013 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 listed in the supplemental data (Appendix 2). The first publication date was fixed on 2004 because the widely used criteria for scoring SD, steatosis, and steatohepatitis were developed in 2004[26] and 2005.[27] No language filter was applied.

Study identification and data collection

Identified studies were listed in EndNote X7. Duplicates were automatically and manually removed. Two authors (JZ, KvM) independently screened all titles and abstracts and excluded those not pertinent. Discrepancy was solved by consensus. The remaining articles were included for full-text revision and independently assessed for eligibility (JZ, KvM). Reference lists of full-text reviewed articles were manually checked for additional potential citations, next to exploration of a personal library (JZ, KvM) that was established because of previous research on this topic.[28,29]

Definitions

SD was graded according to Rubbia-Brandt et al.,[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 >33% of parenchyma affected by steatosis.[27]

A non-alcoholic steatohepatitis activity score (NAS) ≥4 was considered steatohepatitis to provide a working cut-off value in conformity with the literature.[30] CALI was defined as any occurrence of the following: severe SD, severe steatosis, or steatohepatitis. 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 90 days after surgery or during hospital stay and graded according to the classification of Dindo et al.[31] Major morbidity was defined as Dindo-Clavien score III (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.[32] Postoperative liver failure was defined as the concurrent presence of a prothrombin time of less than 50% and a serum bilirubin greater than 50 μM/L on (the "50-50" criteria) or after postoperative day 5.[33] Postoperative mortality was defined as death due to any cause occurring 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

Risk of bias of the final included studies was assessed independently by two blinded researchers (JZ, KvM) 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. For each of six domains, assessment of separate items was taken together to calculate an overall low, moderate, or high risk of bias. The following definitions were chosen for rating the overall risk of bias: 'low' was defined by ≤2 domains rated as moderate risk and the remaining domains as low, 'moderate' was defined by ≥3 domains rated as moderate risk and the remaining domains as low, 'high' was defined as ≥1 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 coded data (by numbering) of 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 and subsequently imported into IBM SPSS Statistics for Windows (version 20.0). 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 ap-

plied, or the 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 (the individual participant data from all studies were pooled and modelled simultaneously). This approach was considered most 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 zero events occurred.[37,38] As for missing values, multiple imputations were performed, assuming missing at random. The number of imputations was determined by the maximum percentage of missing data in the dataset. In this study, 30 imputations were performed, as the maximum percentage of missing data was 26% (minor/major morbidity). Variables in multiple imputations are listed in Appendix 3. Complete case analysis was also conducted for sensitivity analysis. Clustering of patients from different studies was integrated as a separate covariate ('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*-value ≤0.20 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 which did have available data of NAS-subcategories was performed. Analyses of these data were identical to the method described before. Unadjusted and adjusted odds ratios (OR) with 95% confidence intervals (95% CI) were calculated. A *p*-value ≤0.05 was considered significant for all analyses.

RESULTS

The search strategy resulted in 1191 unique hits. 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 respective thirty corresponding authors were contacted by email. Nineteen authors responded, of which eight authors from eight studies agreed to share their raw data. Other authors could not participate because of loss of correspondence. Potential publication bias was excluded by testing for asymmetry in a funnel plot. A flow chart summarizing the inclusion process

Table 1. Characteristics of studies included in final database

Author	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	Ox CTx *vs.* non ox 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	36[‡]	Mod	Neoadjuvant CTx *vs.* CTx + Cet/Bev	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 CALI *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 SD *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[**]	Mod	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	Nether-lands	RCS	104	Low	Neoadjuvant CTx *vs.* CTx + Bev	Bevacizumab added to oxaliplatin-based chemotherapy may protect against moderate sinusoidal dilatation without significantly influencing morbidity
Vigano[17]	2012	Italy	PCS	100	Low	Severe CALI *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

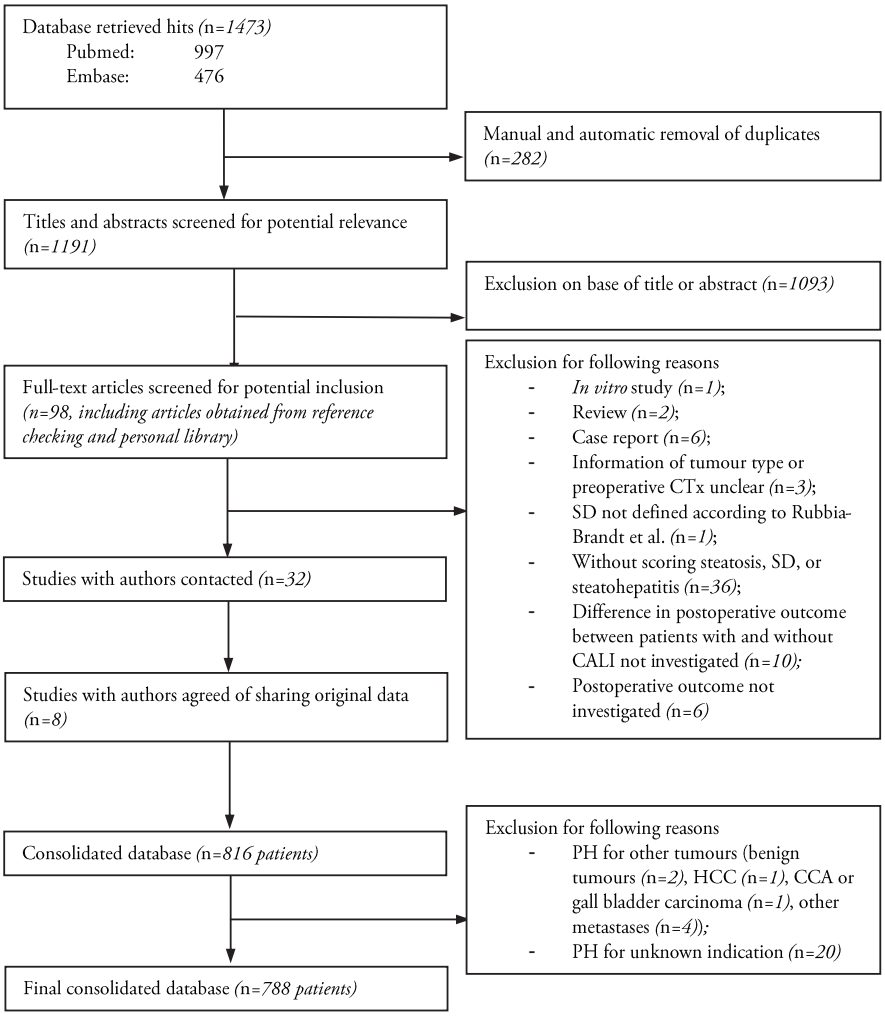
CTx, chemotherapy; SD, sinusoidal dilatation; CALI, chemotherapy-associated liver injury; PH, partial hepatectomy; Bev, bevacizumab

can be found in Appendix 4. The included eight studies were subjected to the QUIPS tool for assessment of risk of bias. Overall ratings of the included studies are depicted in Table 1. Six articles showed an overall low risk of bias and one study each showed a moderate or high risk of bias. Detailed scorings can be found in Appendix 5. Since the individual participant data from all studies were pooled and modelled simultaneously, the domains rating study confounding and statistical analysis were not taken into account for the overall rating. After this consideration, all studies showed low risk of bias on the remaining four domains.

Details of participated studies are depicted in Table 1. The subsequent consolidated cohort consisted of 816 patients. Twenty-eight patients were excluded because they underwent surgery for other indications than CRLM; hence 788 patients were included for analysis. Figure 1 shows a detailed overview of the selection process. Patient characteristics, surgical details, and postoperative outcomes of the consolidated cohort are listed in Table 2. In short, in total 453 (57.5%) patients were male and 335 (42.5%) patients were female with a median age of 61 years (range 25-86). Severe SD was found in 183 (24.1%) patients, severe steatosis in 117 (15.6%) patients, and steatohepatitis in 100 (14.5%) patients. Of the 525 patients that received 5-FU, 396 (75.4%) patients received simultaneous treatment with oxaliplatin and 135 (25.7%) patients with irinotecan. Of the 136 patients that received capecitabine, 119 (87.5%) patients received simultaneous treatment with oxaliplatin. Cetuximab and bevacizumab were most frequently administered together with oxaliplatin. All patients receiving cetuximab (n=61), and 138 of 164 (84.1%) patients receiving bevacizumab, were co-treated with oxaliplatin. Of 635 patients with NAS subcategory data, 96 (15.1%) patients had NAS ≥4. Only 3 (3.1%) patients with NAS ≥4 did not present lobular inflammation. The relationship between NAS and lobular inflammation is summarized in Appendix 6.

Sensitivity analysis showed similar results between complete and multiple imputation case analysis (detailed information available upon 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 depicted in Table 3 and supplemental Figure 1. 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 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 4 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. Within the study cohort, postoperative liver failure occurred in 24 of 779 patients (3.1%) (Table 2). Of these patients, seven were female and 17 were male, with a median age of 61 years (range 48-75). All patients with liver failure had undergone major hepatectomy. Only severe SD and perioperative blood transfusion were strongly associated with increased liver failure in univariable analysis (OR 4.47, 95% CI 1.69-11.82, p=0.003 and OR 3.06, 95% CI 1.18-7.92, p=0.021, respectively).

Table 2. Patient, surgical, and postoperative characteristics

		Sinusoidal dilatation ($n=760$)			Steatosis ($n=752$)			Steatohepatitis ($n=690$)		
	n	Non-severe ($n=577$)	Severe ($n=183$)	P†	Non-severe ($n=635$)	Severe ($n=117$)	P	NAS < 4 ($n=590$)	NAS ≥ 4 ($n=100$)	P†
Age (years)*	788	61(11)	60(10)	0·576‡	61(11)	60(9)	0·527‡	61(11)	61(10)	0·784‡
Sex				0·836			1·000			0·784
M	453	335 (58·1)	104 (56·8)		366 (57·6)	68 (58·1)		336 (56·9)	59 (59·0)	
F	335	242 (41·9)	79 (43·2)		269 (42·4)	49 (41·9)		254 (43·1)	41 (41·0)	
BMI (kg/m^2)*	706	25·3(4·4)	24·4(3·9)	0·001‡	24·7(4·1)	27·6(4·1)	< 0·001‡	24·9(4·2)	26·7(3·9)	< 0·001‡
Co-morbidity				0·259			0·012			0·158
No	363	247 (49·3)	93 (54·7)		290 (52·2)	41 (38·3)		271 (51·4)	31 (42)	
Yes	336	254 (50·7)	77 (45·3)		266 (47·8)	66 (61·7)		256 (48·6)	43 (58)	
Preoperative chemotherapy				0·001			0·032			0·198
No	127	110 (19·3)	15 (8·4)		114 (18·2)	11 (9·6)		112 (19·3)	13 (13)	
Yes	649	459 (80·7)	164 (91·6)		511 (81·8)	104 (90·4)		468 (80·7)	85 (87)	
5-FU				0·006			0·463			0·396
No	245	201 (35·6)	43 (24·2)		210 (34·0)	34 (29·8)		213 (37·0)	31 (32)	
Yes	525	363 (64·4)	135 (75·8)		408 (66·0)	80 (70·2)		362 (63·0)	66 (68)	
Capecitabine				0·173			0·158			0·061
No	634	454 (80·5)	152 (85·4)		511 (82·4)	87 (76·3)		466 (81·0)	70 (72)	
Yes	136	110 (19·5)	26 (14·6)		109 (17·6)	27 (23·7)		109 (19·0)	27 (28)	
Irinotecan				0·033			0·033			1·000
No	627	445 (78·9)	154 (86·5)		508 (81·9)	83 (72·8)		453 (78·8)	77 (79)	
Yes	143	119 (21·1)	24 (13·5)		112 (18·1)	31 (27·2)		122 (21·2)	20 (21)	
Bevacizumab				0·072			0·143			0·101
No	605	430 (76·4)	148 (83·1)		487 (78·7)	82 (71·9)		444 (77·4)	67 (69)	
Yes	164	133 (23·6)	30 (16·9)		132 (21·3)	32 (28·1)		130 (22·6)	30 (31)	
Cetuximab				0·198			0·716			0·462
No	708	522 (92·7)	159 (89·3)		566 (91·4)	106 (93·0)		522 (90·9)	91 (94)	
Yes	61	41 (7·3)	19 (10·7)		53 (8·6)	8 (7·0)		52 (9·1)	6 (6)	
Oxaliplatin				< 0·001			0·716			0·295
No	266	228 (40·5)	37 (20·8)		226 (36·5)	39 (34·2)		231 (40·2)	33 (34)	
Yes	503	335 (59·5)	141 (79·2)		393 (63·5)	75 (65·8)		343 (59·8)	64 (66)	
Resection type				< 0·001			0·921			0·713
Minor (< 3 segments)	398	330 (57·2)	67 (36·8)		335 (52·8)	63 (53·8)		339 (57·6)	55 (55·0)	
Major (≥ 3 segments)	389	247 (42·8)	115 (63·2)		299 (47·2)	54 (46·2)		250 (42·4)	45 (45·0)	
PVE				0·001			0·046			0·512
No	623	461 (92·0)	140 (82·4)		498 (89·6)	103 (96·3)		491 (93·2)	71 (96)	
Yes	76	40 (8·0)	30 (17·6)		58 (10·4)	4 (3·7)		36 (6·8)	3 (4)	
Pringle manoeuvre				0·018			0·974			1·000
No	446	347 (64·5)	93 (54·1)		366 (62·1)	71 (62·8)		349 (63·9)	61 (64)	
Yes	291	191 (35·5)	79 (45·9)		223 (37·9)	42 (37·2)		197 (36·1)	34 (36)	
Transfusion of packed RBCs				0·004			0·871			0·382
No	646	492 (86·2)	139 (76·8)		529 (84·4)	100 (85·5)		508 (86·8)	83 (83·0)	
Yes	134	79 (13·8)	42 (23·2)		98 (15·6)	17 (14·5)		77 (13·2)	17 (17·0)	
Postoperative short-term outcomes										
Length of hospital stay (days)*	768	14(13)	15(11)	0·240‡	15(11)	14(17)	0·660‡	14(12)	15(14)	0·266‡
Overall morbidity				0·009			0·935			0·024
None/minor morbidity (DC 0–II)	491	362 (88·5)	106 (73·1)	< 0·001	397 (85·6)	69 (85)	1·000	367 (89·5)	62 (81)	0·041
Major morbidity (DC III–V)	89	47 (11·5)	39 (26·9)		67 (14·4)	12 (15)		43 (10·5)	15 (19)	
Liver surgery-specific complication				< 0·001			0·031			0·247
No	598	455 (79·8)	121 (66·9)		475 (75·9)	100 (85·5)		470 (80·6)	75 (75·0)	
Yes	180	115 (20·2)	60 (33·1)		151 (24·1)	17 (14·5)		113 (19·4)	25 (25·0)	
Mortality				0·200§			1·000§			0·422§
No	780	573 (99·3)	179 (97·8)		628 (98·9)	116 (99·1)		586 (99·3)	98 (98·0)	
Yes	8	4 (0·7)	4 (2·2)		7 (1·1)	1 (0·9)		4 (0·7)	2 (2·0)	
Postoperative liver failure				< 0·001			0·302§			1·000§
No	755	563 (98·6)	166 (91·7)		608 (97·0)	116 (99·1)		578 (99·0)	99 (99·0)	
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

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 males and two females had a median age of 64 years (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%

Table 3. The influence of liver injury on short-term postoperative outcome

	Overall morbidity				Liver surgery-specific complications			
	Univariable analysis*		Multivariable analysis*		Univariable analysis*		Multivariable analysis*	
Variable	OR [95% CI]	p-value	OR [95% CI]	p-value	OR [95% CI]	p-value	OR [95% CI]	p-value
Gender (female)	0.63 [0.47-0.85]	0.002	0.61 [0.45-0.83]	0.002	0.72 [0.51-1.02]	0.066	0.65 [0.45-0.94]	0.023
Age (years)	1.01 [1.00-1.02]	0.122	1.01 [1.00-1.02]	0.164	0.99 [0.98-1.01]	0.450		
BMI (kg/m2)	0.97 [0.93-1.01]	0.086	0.97 [0.93-1.01]	0.106	0.92 [0.88-0.96]	<0.001	0.93 [0.89-0.98]	0.005
Comorbidity	1.02 [0.57-1.83]	0.950			0.76 [0.30-1.88]	0.537		
PVE	1.85 [1.13-3.05]	0.015	1.31 [0.74-2.32]	0.358	3.84 [2.34-6.31]	<0.001	2.64 [1.46-4.78]	0.001
Preoperative Ctx	0.90 [0.61-1.33]	0.594			0.98 [0.62-1.54]	0.924		
Resection type (major)	2.02 [1.50-2.72]	<0.001	1.72 [1.24-2.40]	0.001	2.42 [1.69-3.48]	<0.001	1.72 [1.14-2.58]	0.010
Pringle manoeuver	1.23 [0.91-1.65]	0.185	0.97 [0.70-1.34]	0.834	1.38 [0.96-1.97]	0.079	0.93 [0.61-1.39]	0.706
Blood transfusion	2.98 [2.01-4.41]	<0.001	2.53 [1.68-3.80]	<0.001	2.17 [1.45-3.26]	<0.001	1.73 [1.10-2.70]	0.017
Severe SD	1.52 [1.09-2.13]	0.014	1.25 [0.86-1.80]	0.239	1.87 [1.29-2.71]	0.001	1.28 [0.83-1.98]	0.268
Severe steatosis	1.03 [0.69-1.54]	0.872			0.52 [0.30-0.90]	0.020	0.52 [0.27-1.00]	0.049
Steatohepatitis	1.67 [1.11-2.51]	0.014	1.58 [0.99-2.52]	0.057	1.61 [0.75-3.46]	0.217	2.08 [1.18-3.66]	0.012†

SD, sinusoidal dilatation; OR, odds ratio; 95% CI, 95% confidence intervals; BMI, body mass index; PVE, portal venous embolization; Ctx, chemotherapy; DC, Dindo-Clavien; *Multiple imputations; †As steatohepatitis was frequently reported as a risk factor for postoperative morbidity and had a *p*-value close to the upper limit for inclusion (0.20), it was forced into multivariable analysis

Table 3. The influence of liver injury on short-term postoperative outcome (continued)

	Major morbidity (DC III-V)			
	Univariable analysis*		Multivariable analysis*	
Variable	OR [95% CI]	p-value	OR [95% CI]	p-value
Gender (female)	1.01 [0.65-1.57]	0.980		
Age (years)	1.02 [0.99-1.04]	0.179	1.02 [0.99-1.04]	0.137
BMI (kg/m^2)	0.99 [0.93-1.05]	0.654		
Comorbidity	1.01 [0.63-1.61]	0.972		
PVE	2.01 [1.11-3.64]	0.021	1.50 [0.76-2.96]	0.246
Preoperative Ctx	1.43 [0.73-2.82]	0.298		
Resection type (major)	1.72 [1.03-2.85]	0.037	1.21 [0.67-2.22]	0.526
Pringle manoeuver	1.38 [0.86-2.19]	0.179	1.12 [0.67-1.88]	0.667
Blood transfusion	3.28 [2.01-5.35]	**<0.001**	2.77 [1.66-4.61]	**<0.001**
Severe SD	2.09 [1.30-3.38]	0.003	1.73 [1.02-2.95]	0.043
Severe steatosis	1.07 [0.55-2.09]	0.835		
Steatohepatitis	1.85 [0.94-3.63]	0.073	1.67 [0.85-3.30]	0.139

SD, sinusoidal dilatation; OR, odds ratio; 95% CI, 95% confidence intervals; BMI, body mass index; PVE, portal venous embolization; Ctx, chemotherapy; DC, Dindo-Clavien; *Multiple imputations

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 an increased occurrence of postoperative overall morbidity (OR 2.22, 95% CI 1.48-3.34, p=0.001), liver surgery-specific morbidity (OR 3.35, 95% CI 2.21-5.32, 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.

Lastly, Table 5 and supplemental Figure 2 summarize the association between several preoperative variables and the occurrence of severe SD, severe steatosis, and steatohepatitis. Oxaliplatin (OR 2.74, 95% CI 1.67-4.49, p<0.001) was related to an 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 solely adjusted by database

Table 4. Factors influencing short-term postoperative outcome

	Postoperative liver failure (n=24)		Mortality (n=8)	
	Univariable analysis*		Univariable analysis*	
Variable	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/m2)	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 manoeuver	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; ‡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)

source. Furthermore, 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). Body-mass index (BMI) was related to an increase in severe steatosis (OR 1.15, 95% CI 1.08-1.21, p<0.001), whereas a decreased incidence of severe steatosis was seen 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).

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

Table 5. Factors related to liver injury

	Severe SD				Severe steatosis				Steatohepatitis			
	Univariable analysis*		Multivariable analysis*		Univariable analysis*		Multivariable analysis*		Univariable analysis*		Multivariable analysis*	
Variable	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
Gender (female)	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		
Comorbidity	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	n/a	n/a	n/a	n/a	14.52 [6.07-34.71]	**<0.001**	15.09 [6.25-36.45]	**<0.001**
Severe SD	n/a	n/a	n/a	n/a	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**	n/a	n/a	n/a	n/a

BMI, body mass index; PVE, portal venous embolization; 5-FU, 5-Fluorouracil; SD, sinusoidal dilatation; OR, odds ratio; 95% CI, 95% confidence intervals; n/a, not applicable; *Multiple imputations; [†]As irinotecan was frequently reported as a risk factor for steatohepatitis in literature, it was forced into multivariable analysis

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 remodelling 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-registry[59] provide a way to obtain a higher level of evidence on this topic.

ACKNOWLEDGMENTS

We would like to thank Ms. Hang Nguyen for thorough reading and commenting on the manuscript.

REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; **136**:359-386.
2. Leonard GD, Brenner B, Kemeny NE. Neoadjuvant chemotherapy before liver resection for patients with unresectable liver metastases from colorectal carcinoma. *J Clin Oncol* 2005; **23**:2038-2048.
3. Kanas GP, Taylor A, Primrose JN, Langeberg WJ, Kelsh MA, Mowat FS et al. Survival after liver resection in metastatic colorectal cancer: review and meta-analysis of prognostic factors. *Clin Epidemiol* 2012; **4**:283-301.
4. Scheele J. Hepatectomy for liver metastases. *Br J Surg* 1993; **80**:274-276.
5. Tanaka K, Adam R, Shimada H, Azoulay D, Levi F, Bismuth H. Role of neoadjuvant chemotherapy in the treatment of multiple colorectal metastases to the liver. *Br J Surg* 2003; **90**:963-969.
6. Adam R, Wicherts DA, de Haas RJ, Ciacio O, Levi F, Paule B et al. Patients with initially unresectable colorectal liver metastases: is there a possibility of cure? *J Clin Oncol* 2009; **27**:1829-1835.
7. Nordlinger B, Van Cutsem E, Rougier P, Kohne CH, Ychou M, Sobrero A et al. Does chemotherapy prior to liver resection increase the potential for cure in patients with metastatic colorectal cancer? A report from the European Colorectal Metastases Treatment Group. *Eur J Cancer* 2007; **43**:2037-2045.
8. Robinson SM, Wilson CH, Burt AD, Manas DM, White SA. Chemotherapy-associated liver injury in patients with colorectal liver metastases: a systematic review and meta-analysis. *Ann Surg Oncol* 2012; **19**:4287-4299.
9. Zorzi D, Laurent A, Pawlik TM, Lauwers GY, Vauthey JN, Abdalla EK. Chemotherapy-associated hepatotoxicity and surgery for colorectal liver metastases. *Br J Surg* 2007; **94**:274-286.
10. Rubbia-Brandt L, Lauwers GY, Wang H, Majno PE, Tanabe K, Zhu AX et al. Sinusoidal obstruction syndrome and nodular regenerative hyperplasia are frequent oxaliplatin-associated liver lesions and partially prevented by bevacizumab in patients with hepatic colorectal metastasis. *Histopathology* 2010; **56**:430-439.
11. Vigano L, Rubbia-Brandt L, De Rosa G, Majno P, Langella S, Toso C et al. Nodular Regenerative Hyperplasia in Patients Undergoing Liver Resection for Colorectal Metastases After Chemotherapy: Risk Factors, Preoperative Assessment and Clinical Impact. *Ann Surg Oncol* 2015; **22**:4149-4157.
12. Ribero D, Wang H, Donadon M, Zorzi D, Thomas MB, Eng C et al. Bevacizumab improves pathologic response and protects against hepatic injury in patients treated with oxaliplatin-based chemotherapy for colorectal liver metastases. *Cancer* 2007; **110**:2761-2767.
13. Vauthey JN, Pawlik TM, Ribero D, Wu TT, Zorzi D, Hoff PM et al. Chemotherapy regimen predicts steatohepatitis and an increase in 90-day mortality after surgery for hepatic colorectal metastases. *J Clin Oncol* 2006; **24**:2065-2072.
14. Khan AZ, Morris-Stiff G, Makuuchi M. Patterns of chemotherapy-induced hepatic injury and their implications for patients undergoing liver resection for colorectal liver metastases. *J Hepatobiliary Pancreat Surg* 2009; **16**:137-144.
15. Nakano H, Oussoultzoglou E, Rosso E, Casnedi S, Chenard-Neu MP, Dufour P et al. Sinusoidal injury increases morbidity after major hepatectomy in patients with colorectal liver metastases receiving preoperative chemotherapy. *Ann Surg* 2008; **247**:118-124.
16. Pilgrim CH, Satgunaseelan L, Pham A, Murray W, Link E, Smith M et al. Correlations between histopathological diagnosis of chemotherapy-induced hepatic injury, clinical features, and perioperative morbidity. *HPB* 2012; **14**:333-340.

17. Vigano L, Ravarino N, Ferrero A, Motta M, Torchio B, Capussotti L. Prospective evaluation of accuracy of liver biopsy findings in the identification of chemotherapy-associated liver injuries. *Arch Surg* 2012; **147**:1085-1091.
18. Aloia T, Sebagh M, Plasse M, Karam V, Levi F, Giacchetti S et al. Liver histology and surgical outcomes after preoperative chemotherapy with fluorouracil plus oxaliplatin in colorectal cancer liver metastases. *J Clin Oncol* 2006; **24**:4983-4990.
19. Komori H, Beppu T, Baba Y, Horino K, Imsung C, Masuda T et al. Histological liver injury and surgical outcome after FOLFOX followed by a hepatectomy for colorectal liver metastases in Japanese patients. *Int J Clin Oncol* 2010; **15**:263-270.
20. Pawlik TM, Olino K, Gleisner AL, Torbenson M, Schulick R, Choti MA. Preoperative chemotherapy for colorectal liver metastases: impact on hepatic histology and postoperative outcome. *J Gastrointest Surg* 2007; **11**:860-868.
21. Sahajpal A, Vollmer CM, Dixon E, Chan EK, Wei A, Cattral MS et al. Chemotherapy for colorectal cancer prior to liver resection for colorectal cancer hepatic metastases does not adversely affect perioperative outcomes. *J Surg Oncol* 2007; **95**:22-27.
22. Makowiec F, Mohrle S, Neeff H, Drognitz O, Illerhaus G, Opitz OG et al. Chemotherapy, liver injury, and postoperative complications in colorectal liver metastases. *J Gastrointest Surg* 2011; **15**:153-164.
23. Kandutsch S, Klinger M, Hacker S, Wrba F, Gruenberger B, Gruenberger T. Patterns of hepatotoxicity after chemotherapy for colorectal cancer liver metastases. *Eur J Surg Oncol* 2008; **34**:1231-1236.
24. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med* 2009; **151**:264-269.
25. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA* 2000; **283**:2008-2012.
26. Rubbia-Brandt L, Audard V, Sartoretti P, Roth AD, Brezault C, Le Charpentier M et al. Severe hepatic sinusoidal obstruction associated with oxaliplatin-based chemotherapy in patients with metastatic colorectal cancer. *Ann Oncol* 2004; **15**:460-466.
27. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005;**41**:1313-1321.
28. Vreuls CP, Van Den Broek MA, Winstanley A, Koek GH, Wisse E, Dejong CH et al. Hepatic sinusoidal obstruction syndrome (SOS) reduces the effect of oxaliplatin in colorectal liver metastases. *Histopathology* 2012; **61**:314-318.
29. van Mierlo KMC, Zhao J, Kleijnen J, Rensen SS, Schaap FG, Dejong CHC et al. The influence of chemotherapy-associated sinusoidal dilatation on short-term outcome after partial hepatectomy for colorectal liver metastases: A systematic review with meta-analysis. *Surg Oncol* 2016; **25**:298-307.
30. Brunt EM, Kleiner DE, Wilson LA, Belt P, Neuschwander-Tetri BA, Network NCR. Nonalcoholic fatty liver disease (NAFLD) activity score and the histopathologic diagnosis in NAFLD: distinct clinicopathologic meanings. *Hepatology* 2011; **53**:810-820.
31. Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. *Ann Surg* 2004; **240**:205-213.
32. van den Broek MA, van Dam RM, van Breukelen GJ, Bemelmans MH, Oussoultzoglou E, Pessaux P et al. Development of a composite endpoint for randomized controlled trials in liver surgery. *Br J Surg* 2011; **98**:1138-1145.
33. Balzan S, Belghiti J, Farges O, Ogata S, Sauvanet A, Delefosse D et al. The "50-50 criteria" on postoperative day 5: an accurate predictor of liver failure and death after hepatectomy. *Ann Surg* 2005; **242**:824-828.

34. Couinaud C. Liver anatomy: portal (and suprahepatic) or biliary segmentation. *Dig Surg* 1999; **16**:459-467.
35. Hayden JA, van der Windt DA, Cartwright JL, Cote P, Bombardier C. Assessing bias in studies of prognostic factors. *Ann Intern Med* 2013; **158**:280-286.
36. Hayden JA, Cote P, Bombardier C. Evaluation of the quality of prognosis studies in systematic reviews. *Ann Intern Med* 2006; **144**:427-437.
37. Debray TP, Moons KG, Abo-Zaid GM, Koffijberg H, Riley RD. Individual participant data meta-analysis for a binary outcome: one-stage or two-stage? *PLoS One* 2013; **8**:60650.
38. Abo-Zaid G, Guo B, Deeks JJ, Debray TP, Steyerberg EW, Moons KG et al. Individual participant data meta-analyses should not ignore clustering. *J Clin Epidemiol* 2013; **66**:865-873.
39. Schiffer E, Frossard JL, Rubbia-Brandt L, Mentha G, Pastor CM. Hepatic regeneration is decreased in a rat model of sinusoidal obstruction syndrome. *J Surg Oncol* 2009; **99**:439-446.
40. Hubert C, Dahrenmoller C, Marique L, Jabbour N, Gianello P, Leclercq I. Hepatic regeneration in a rat model is impaired by chemotherapy agents used in metastatic colorectal cancer. *Eur J Surg Oncol* 2015; **41**:1471-1478.
41. Sanyal AJ, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK et al. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology* 2001; **120**:1183-1192.
42. Cortez-Pinto H, Chatham J, Chacko VP, Arnold C, Rashid A, Diehl AM. Alterations in liver ATP homeostasis in human nonalcoholic steatohepatitis: a pilot study. *JAMA* 1999; **282**:1659-1664.
43. Yang SQ, Lin HZ, Mandal AK, Huang J, Diehl AM. Disrupted signaling and inhibited regeneration in obese mice with fatty livers: implications for nonalcoholic fatty liver disease pathophysiology. *Hepatology* 2001; **34**:694-706.
44. Wiggans MG, Lordan JT, Shahtahmassebi G, Aroori S, Bowles MJ, Stell DA. The Interaction between Diabetes, Body Mass Index, Hepatic Steatosis, and Risk of Liver Resection: Insulin Dependent Diabetes Is the Greatest Risk for Major Complications. *HPB Surg* 2014; **2014**:586159.
45. Reddy SK, Marsh JW, Varley PR, Mock BK, Chopra KB, Geller DA et al. Underlying steatohepatitis, but not simple hepatic steatosis, increases morbidity after liver resection: a case-control study. *Hepatology* 2012; **56**:2221-2230.
46. McCormack L, Petrowsky H, Jochum W, Furrer K, Clavien PA. Hepatic steatosis is a risk factor for postoperative complications after major hepatectomy: a matched case-control study. *Ann Surg* 2007; **245**:923-930.
47. Kooby DA, Fong Y, Suriawinata A, Gonen M, Allen PJ, Klimstra DS et al. Impact of steatosis on perioperative outcome following hepatic resection. *J Gastrointest Surg* 2003; **7**:1034-1044.
48. van der Pool AE, Marsman HA, Verheij J, Ten Kate FJ, Eggermont AM, IJzermans JN et al. Effect of bevacizumab added preoperatively to oxaliplatin on liver injury and complications after resection of colorectal liver metastases. *J Surg Oncol* 2012; **106**:892-897.
49. Rubbia-Brandt L, Tauzin S, Brezault C, Delucinge-Vivier C, Descombes P, Dousset B et al. Gene expression profiling provides insights into pathways of oxaliplatin-related sinusoidal obstruction syndrome in humans. *Mol Cancer Ther* 2011; **10**:687-696.
50. Farrell GC, Teoh NC, McCuskey RS. Hepatic microcirculation in fatty liver disease. *Anat Rec (Hoboken)* 2008; **291**:684-692.
51. Brouquet A, Benoist S, Julie C, et al. Risk factors for chemotherapy-associated liver injuries: A multivariate analysis of a group of 146 patients with colorectal metastases. *Surgery* 2009; **145**:362-371.

52. Kollmar O, Corsten M, Scheuer C, Vollmar B, Schilling MK, Menger MD. Portal branch ligation induces a hepatic arterial buffer response, microvascular remodeling, normoxygenation, and cell proliferation in portal blood-deprived liver tissue. *Am J Physiol Gastrointest Liver Physiol* 2007; **292**:1534-1542.
53. Matsuo K, Murakami T, Kawaguchi D, et al. Histologic features after surgery associating liver partition and portal vein ligation for staged hepatectomy versus those after hepatectomy with portal vein embolization. *Surgery* 2016; **159**:1289-1298.
54. van Mierlo KM, Schaap FG, Dejong CH, Olde Damink SW. Liver resection for cancer: New developments in prediction, prevention and management of postresectional liver failure. *J Hepatol* 2016; **65**:1217-1231
55. Farid SG, White A, Khan N, Toogood GJ, Prasad KR, Lodge JP. Clinical outcomes of left hepatic trisectionectomy for hepatobiliary malignancy. *Br J Surg* 2016; **103**:249-256.
56. Vigano L, Capussotti L, De Rosa G, De Saussure WO, Mentha G, Rubbia-Brandt L. Liver Resection for Colorectal Metastases after Chemotherapy Impact of Chemotherapy-Related Liver Injuries, Pathological Tumor Response, and Micrometastases on Long-term Survival. *Ann Surg* 2013; **258**:731-742.
57. Tamandl D, Klinger M, Eipeldauer S, Herberger B, Kaczirek K, Gruenberger B et al. Sinusoidal obstruction syndrome impairs long-term outcome of colorectal liver metastases treated with resection after neoadjuvant chemotherapy. *Ann Surg Oncol* 2011; **18**:421-430.
58. Khoo E, O'Neill S, Brown E, Wigmore SJ, Harrison EM. Systematic review of systemic adjuvant, neoadjuvant and perioperative chemotherapy for resectable colorectal-liver metastases. *HPB* 2016; **18**:485-493.
59. Schadde E, Ardiles V, Robles-Campos R, Malago M, Machado M, Hernandez-Alejandro R et al. Early survival and safety of ALPPS: first report of the International ALPPS Registry. *Ann Surg* 2014; **260**:829-836.
60. Gomez-Ramirez J, Martin-Perez E, Amat CG, Sanz IG, Bermejo E, Rodriguez A et al. Influence of pre-surgical chemotherapy on liver parenchyma and post-surgical outcome of patients subjected to hepatectomy due to colorectal carcinoma metastases. *Cir Esp* 2010; **88**:404-412.
61. Nam SJ, Cho JY, Lee HS, Choe G, Jang JJ, Yoon YS et al. Chemotherapy-associated hepatopathy in korean colorectal cancer liver metastasis patients: oxaliplatin-based chemotherapy and sinusoidal injury. *Korean J Pathol* 2012; **46**:22-29.
62. Pessaux P, Marzano E, Casnedi S, Bachellier P, Jaeck D, Chenard MP. Histological and immediate postoperative outcome after preoperative cetuximab: case-matched control study. *World J Surg* 2010; **34**:2765-2772.
63. Soubrane O, Brouquet A, Zalinski S, Terris B, Brezault C, Mallet V et al. Predicting high grade lesions of sinusoidal obstruction syndrome related to oxaliplatin-based chemotherapy for colorectal liver metastases: correlation with post-hepatectomy outcome. *Ann Surg* 2010; **251**:454-460.
64. Takamoto T, Hashimoto T, Sano K, Maruyama Y, Inoue K, Ogata S et al. Recovery of liver function after the cessation of preoperative chemotherapy for colorectal liver metastasis. *Ann Surg Oncol* 2010; **17**:2747-2755.

CALI consortium

Name and title	Affiliation
Junfang Zhao, MD	Department of Surgery, Maastricht University Medical Centre & NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University, Maastricht, the Netherlands
Kim M.C. van Mierlo, MD	
Sander S. Rensen, PhD	
Frank G. Schaap, PhD	
Prof. Cornelis H.C. Dejong, MD, PhD	Department of Surgery, Maastricht University Medical Centre & NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University, Maastricht, the Netherlands; GROW School for Oncology and Developmental Biology, Maastricht University, Maastricht, the Netherlands
Prof. Steven W.M. Olde Damink, MD, PhD	Department of Surgery, Maastricht University Medical Centre & NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University, Maastricht, the Netherlands; Department of Hepato-pancreatico-biliary Surgery & Liver Transplantation, Institute for Liver and Digestive Health, Royal Free Hospitals, University College London, London, United Kingdom
Joaquín Gómez-Ramírez, MD, PhD	Hepato-pancreatico-biliary Surgery Unit, Department of Surgery, Hospital Universitario de la Princesa, Madrid, Spain
Iñigo García Sanz, MD	Hepato-pancreatico-biliary Surgery Unit, Department of Surgery, Hospital Universitario de la Princesa, Madrid, Spain
Elena Martín Pérez, MD, PhD	Hepato-pancreatico-biliary Surgery Unit, Department of Surgery, Hospital Universitario de la Princesa, Madrid, Spain
Haeryoung Kim, MD, PhD	Department of Pathology, Seoul National University Bundang Hospital, Seoul National College of Medicine, Seongnam, Korea
Prof. Jai Young Cho, MD, PhD	Department of Surgery, Seoul National University Bundang Hospital, Seoul National College of Medicine, Seongnam, Korea
Prof. Young Rok Choi, MD, PhD	Department of Surgery, Seoul National University Bundang Hospital, Seoul National College of Medicine, Seongnam, Korea
Charles H.C. Pilgrim, MBBS (Hons), PhD	Hepato-pancreatico-biliary Service, Upper Gastrointestinal Surgery, The Alfred Hospital, Melbourne, Australia; Division of Cancer Surgery, Peter MacCallum Cancer Centre, Department of Surgery, University of Melbourne, St Vincent's Hospital
Prof. Wayne Phillips, MD, PhD	Division of Cancer Surgery, Peter MacCallum Cancer Centre; Department of Surgery, University of Melbourne, St. Vincent's Hospital, Melbourne, Australia
Prof. Michael Michael, MD, PhD	Division of Cancer Medicine, Peter MacCallum Cancer Centre; Department of Medicine, University of Melbourne, St. Vincent's Hospital, Melbourne, Australia
Prof. Patrick Pessaux, MD, PhD	Digestive surgery and transplantation, Hôpital de Hautepierre, University hospital of Strasbourg, Strasbourg, France
Prof. Fabrizio Panaro, MD, PhD	Digestive surgery and transplantation, Hôpital de Hautepierre, University hospital of Strasbourg, University of Strasbourg, Strasbourg, France
Prof. Marie-Pierre Chenard, MD, PhD	Department of Pathology, Hôpital de Hautepierre, University hospital of Strasbourg, University of Strasbourg, Strasbourg, France
Eric P. van der Stok, MD	Department of Surgical Oncology, Erasmus MC Cancer institute, Rotterdam, the Netherlands
Prof. Cornelis Verhoef, MD, PhD	Department of Surgical Oncology, Erasmus MC Cancer institute, Rotterdam, the Netherlands

Dirk J. Grünhagen, MD, PhD	Department of Surgical Oncology, Erasmus MC Cancer institute, Rotterdam, the Netherlands
Prof. Olivier Soubrane, MD, PhD	Department of Hepato-biliary Surgery and Liver Transplant, Beaujon Hospital, Assistance Publique - Hôpitaux de Paris, Université Denis Diderot, Paris, France
Jeremy Vara, MD	Digestive Tumours Unit, Institut Bergonié, Bordeaux, France.
Prof. Olivier Scatton, MD, PhD	Department of Digestive and Hepato-biliary Surgery, La Pitié Hospital, Université Pierre et Maris Curie, Paris, France
Prof. Takeshi Takamoto, MD, PhD	Department of Hepato-pancreatico-biliary Surgery, Japanese Red Cross Medical Centre, Tokyo, Japan
Takuya Hashimoto, MD, PhD	Department of Hepato-pancreatico-biliary Surgery, Japanese Red Cross Medical Centre, Tokyo, Japan
Masatoshi Makuuchi, MD, PhD	Department of Hepato-pancreatico-biliary Surgery, Japanese Red Cross Medical Centre, Tokyo, Japan
Luca Viganò, MD, PhD	Division of Hepato-biliary & General Surgery, Humanitas Clinical and Research Centre, Humanitas University, Rozzano, Italy
Giovanni De Rosa, MD	Department of Pathology, Mauriziano Umberto I Hospital, Torino, Italy
Nicoletta Ravarino, MD	Department of Pathology, Mauriziano Umberto I Hospital, Torino, Italy

SUPPLEMENTAL DATA

Appendix 1 is available online via: doi: 10.1002/bjs.10572

Appendix 2. Search strategy

Category 1st	Category 2nd	Category 3rd	Category 4th	Filters
Liver resection	**Chemotherapy**	**Tumour type**	**Outcomes**	
Liver resection OR Liver transection OR Hepatic transection OR Hepatectomy AND	Chemotherap* OR Oxaliplatin OR Irinotecan OR FOLFOX OR FOLFIRI OR CAPOX OR 5FU OR Fluorouracil AND	Colorectal neoplasm OR Colorectal cancer OR Colorectal metastas* OR Colon cancer Liver metastas* AND	Morbidity OR Mortality OR Survival OR Dead OR Outcome OR Recurrence OR Liver failure OR Regeneration OR Hepatic insufficiency	Dates: Jan 1, 2004 – Dec 31, 2013 Species: human Age: >18 years

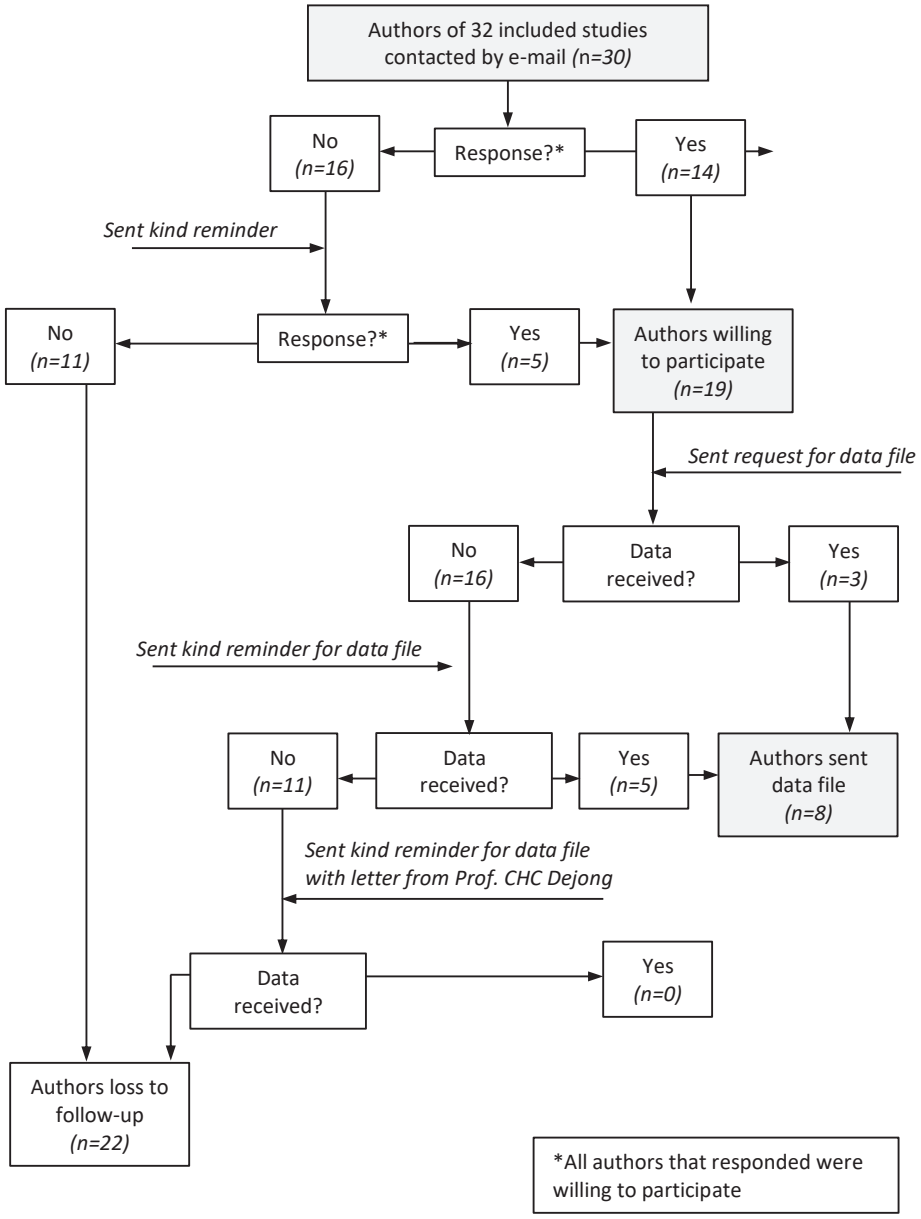
Asterisks indicate blurred searching in case of synonymous words

Appendix 3. Variables in multiple imputations

Patient cluster	General	Chemotherapy	Liver injury	Surgical factors	Outcome
Database source	Gender, age, BMI, comorbidity	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

BMI, body mass index; PVE, portal venous embolization; RBC, red blood cells

Appendix 4. Flow chart inclusion process



Appendix 5. Quality in prognostic studies tool (QUIPS) detailed risk of bias assessment of individual included studies

	Gómez-Ramírez[60]	Nam[61]	Pessaux[62]	Pilgrim[16]	Soubrane[63]	Takamoto[64]	Van der Pool[48]	Viganò[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	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
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
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
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	✓	✓	✓	✓	✓⁻	✓	✓	✓

	Gómez-Ramírez[60]	Nam[61]	Pessaux[62]	Pilgrim[16]	Soubrane[63]	Takamoto[64]	Van der Pool[48]	Viganò[17]
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

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

Appendix 6. Cross tabulation showing the relationship between NAS and lobular inflammation

	Lobular inflammation				
NAS	**No foci**	**<2 foci per 200x field**	**2–4 foci per 200x field**	**>4 foci per 200x field**	**Total number of patients**
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 number of patients	192	308	108	27	635

NAS, non-alcoholic fatty liver disease activity score

PART II

Postoperative detection of liver dysfunction

Chapter 5

Surrogate endpoints in liver surgery related trials: a systematic review of the literature

Liliane Mpabanzi, Kim M.C. van Mierlo, Massimo Malagó, Cornelis H.C. Dejong, Dimitrios Lytras, Steven W.M. Olde Damink

HPB (Oxford). 2013 May;15(5):327-36

ABSTRACT

Background. Although the safety of liver surgery has improved enormously, hepatic surgery continues to face challenging complications. Therefore, improvements supported by evidence-based guidelines are still required. The conduct of randomized controlled trials in liver surgery using dichotomous outcomes requires a large sample size. The use of surrogate endpoints (SEPs) reduces sample size but SEPs should be validated before use. The aim of this review was to summarize the SEPs used in hepatic surgery related trials, their definitions and recapitulating the evidence validating their use.

Methods. A systematic computerized literature search in the biomedical database PubMed using the MeSH terms 'hepatectomy' or 'liver resection' or 'liver transection' was conducted. Search was limited to papers written in the English language and published between 1 January 2000 and 1 January 2010.

Results. A total of 593 articles met the search terms and 49 articles were included in the final selection. Standard biochemical liver functions tests were the most frequently used SEP (32 of 49 the studies). The used definitions of SEPs varied greatly among the studies. Most studies referred to earlier published material to justify their choice of SEP. However, no validating studies were found.

Conclusion. Many SEPs are used in liver surgery trials however there is little evidence validating them.

INTRODUCTION

In the last decades, liver surgery has been a constantly evolving field and its prominent role in the treatment of primary, secondary, malignant or non-malignant liver diseases has been well established.[1] Although considerable improvements in mortality and morbidity rates have been achieved in many surgical centres, complications as a result of surgically induced liver damage still represent challenging events.[2] Consequently, trials evaluating surgical techniques and therapeutic interventions with appropriate endpoints are still needed in this field.

Randomized controlled trials (RCTs) are considered mandatory in evaluating the significance of clinical interventions and their potential implementation in daily clinical practice.[3] However, conducting adequately powered RCTs is frequently not feasible in many medical fields such as hepatic surgery.[4-6] In spite of calls for more rigorous surgical research trials, the overall number and quality of RCTs in surgery remains suboptimal.[6] The introduction of standards in reporting RCTs such as the Consolidated Standards of Reporting Trials (CONSORT)[7] has led to the necessity of defining primary and, if required, secondary outcomes.[7] Thus, it is imperative to standardize the definitions of the endpoints reported in hepatic surgery trials. Currently, the chosen outcomes are mostly clinical endpoints and can be divided in short- (e.g., peri-operative complications and peri-operative or 30 days mortality) and long-term outcomes (e.g., survival and disease-free survival). Van den Broek et al. recently demonstrated that conducting an adequately powered RCT in liver surgery using the clinical dichotomous outcomes mortality and morbidity was not feasible because of the required large sample size.[8] The introduction of surrogate endpoints (SEPs) in RCTs is considered a potential solution for solving the problems which usually compromise the conduct of a sound trial such as complexity, sample size, long-term follow-up, and costs.[9]

A SEP is a laboratory measurement or a physical sign used as a substitute for a clinically meaningful endpoint that measures directly how a patient feels, functions or survives.[10-12] Ideally, changes on a SEP induced by a therapy should reflect a clinically meaningful endpoint. In practice, this requirement frequently fails.[13] Moreover, SEPs should be validated before being used to assess clinical outcomes.

Nonetheless, validation is usually overlooked, especially if biologically plausible grounds are given.[14] In practice, a correlation is often considered as validation for a SEP. However, it has been demonstrated that a correlate does not make a surrogate.[13]

The aim of the present systematic review was to summarize the SEPs representing the effect of surgically induced damage used in liver surgery trials. Additionally, this study aimed at finding common definitions of the used SEPs and at recapitulating the evidence or validation justifying the use of those endpoints.

METHODS

Search strategy

Three authors (KvM., DL and SOD) performed a systematic computerized literature search according to the methodology recommended by the Cochrane Collaboration. The worldwide database of biomedical literature PubMed was searched using the Medical Subject headings (MeSH) terms: 'hepatectomy' or 'liver resection' or 'liver transection'. Additionally, the three authors (LM, KvM and DL) manually reviewed all the articles' references lists for identification of relevant studies. Search results were stored in an Endnote file (Endnote X2, Philadelphia, PA, USA).

Study eligibility

The search was limited to patients older than 18 years, and to articles published between 1 January 2000 and 1 January 2010. Exclusion criteria were non-human studies and papers published in languages other than English. Studies were eligible if they used SEPs as primary or secondary outcome measures. They were excluded if they reported only on dichotomous outcomes, such as mortality and morbidity, or only reported on survival. Papers using outcome measures that were not considered as surrogate markers for liver injury, i.e., the need for a blood transfusion, the amount of operative blood loss or transection time, were also excluded as well as studies reporting on surgical procedures other than liver resection or assessing the effects of different therapeutic modalities such as chemotherapy, radio-frequency ablation, or liver transplantation. Trials focusing on non-surgical interventions such as imaging, the effect of portal vein embolization as well as on liver regeneration were also excluded.

Search of evidence justifying the use of SEPs

All included studies were further scrutinized for references justifying the choice of the used SEPs. All references related to the endpoints mentioned either in the 'introduction' or in the 'materials and methods' sections were considered as references providing evidence and justification for the choice of the SEP. All references were assessed for compliance with the Boissel criteria for validation of SEPs.[15] Briefly, SEPs were checked upon three criteria: convenience, prediction validity and relationship with clinical endpoint.

Data collection and analysis

Two authors (KvM and LM) extracted the data, and the results were reviewed independently by two other authors (DL and SOD). Disagreements were solved by discussion. The following data were recorded systematically after formation of the final list of studies fulfilling the inclusion criteria: first author, year of publication, study design, number of patients, defined endpoints, and the association of defined endpoints with morbidity, mortality, or survival. The references given by the authors to explain the choice of the surrogate endpoints were also recorded in a separate Endnote file.

RESULTS

Quality and quantity of evidence

A total of 593 articles met the search terms. Overall, 552 were excluded. Of those, 320 were excluded after reading the abstract and 232 were excluded after reading the full text. Cross-checking through the references of the included papers delivered an additional 8 studies, resulting in a total of 49 articles being included in this review (Figure 1). All studies were either RCTs or consecutive case series. The search for references justifying the choice of endpoints delivered 125 articles. These studies were mainly reviews or previously published clinical trials (data not shown).

Used SEPs and their definitions

Several biomarkers of hepatic functions as well as systemic parameters were used as SEPs (Table 1). Standard biochemical liver functions tests to quantify hepatocellular damage (post-operative plasma alanine transaminase (ALT), aspartate transaminase (AST), gamma glutamyl transpeptidase (GGT) and alkaline phosphatase (AP)) were the most frequently used SEPs (32 of the 49 studies). Hepatic synthetic function quantified with various haematological factors such as prothrombin time (PT) and platelet count was also frequently used (29 studies). Only two studies used a single SEP. The remaining studies examined a combination of two or more SEPs. Eleven studies did not find a correlation between the used SEP and a clinical outcome.

The definitions of the used SEPs varied greatly among studies (Table 2). The most frequently used SEPs (biochemical liver function tests) were often defined as plasma peak values over a period of 3 to 7 days post-operatively. These discrepancies in definitions and timing of measurement of the SEPs were seen throughout the studies included in this review (Table 2). Authors aimed at showing a correlation between clinical outcomes (mortality and/or morbidity) or an independent predicting factor of these dichotomous outcomes.

Evidence justifying the choice of SEPs

A total of 26 studies referred to earlier published studies to support their choice of SEP (Table 3). From the retrieved studies justifying the SEP, 46% were experimental studies and 77% were clinical studies. Only 6 studies used RCTs as a reference for the selection of their endpoint. Of these none was a validating study for a SEP.

Validation of the SEPs using the three criteria defined by Boissel et al.

The used SEPs occurred more often than clinical endpoints, therefore complying to the first criterium described by Boissel et al.[15] The two other criteria of Boissel et al. could not be verified in this review as they required full insight of the original data and potential follow-up of the patients.

DISCUSSION

The present systematic literature review is an attempt at a comprehensive and critical evaluation of surrogate endpoints used in liver surgery-related clinical trials. Most studies used biological plausible, though not validated, surrogate outcomes. As the liver is involved in a multitude of processes, many functions could serve as surrogate endpoints. In line with this, many surrogate outcomes have been used in reporting the results of trials in hepatic surgery. However, there was a lack in standardized definitions of the most used SEPs.

Liver surgery has been a subject of extensive research in the past two decades.[16] As a result, the safety and efficacy of surgical interventions have increased substantially while the indications for performing a liver resection are continuously extending.[17-20] In spite of a decrease in mortality and morbidity rates, there is still a need for standardized outcome parameters to evaluate therapeutic efficacy or hazards of liver operations.[21] Composite and surrogate outcomes are considered as statistically adequate alternatives for replacing the standard short-term dichotomous outcome of mortality and morbidity in many medical fields.[21-25] Formulation of surrogate outcomes requires, first and foremost, standardization of definitions of the used SEPs. The lack of adequate definitions of outcomes impairs comparison and evaluation of diagnostic and therapeutic trials. Recently, van den Broek et al.[21] conducted a survey among hepato-biliary surgeons to reach a consensus on definitions of complications after liver surgery. These definitions were extracted from the currently available literature and standardized by the authors before being subjected to discussion by experts. The need for the aforementioned survey on definitions was because of the lack of uniformity on definitions as shown in the present review. To reach a consensus on the definitions of the SEPs frequently used in liver surgery related trials, a questionnaire

similar to the survey above stated should be designed. Defining SEPs for hepatic surgery trials should consider the numerous targets of interventions in liver surgery related trials. As all the currently used SEPs are yet to be validated, many definitions can be proposed and adapted to the different effects expected from various interventions.

In the present comprehensive literature review, we were able to retrieve references rationalizing the selection of SEPs used in the majority of the studies. These references were studies using similar endpoints either in clinical trials or in experimental settings. Unfortunately, no study using validated SEPs was found. Validation and value of SEPs has been, and still is, a matter of debate.[13,26] Prentice developed four criteria that are sufficient to validate a SEP in phase three clinical trials.[12] However, these criteria have been considered too stringent and difficult to verify.[27,28] In a comprehensive review, Boissel et al.[15] redefined the three main criteria that a SEP must meet to be considered as valid surrogate for a clinical endpoint. First, a surrogate should be convenient, i.e., it should occur more often than the corresponding clinical point. The time course of the SEP should precede that of the clinical endpoint so that disease or its progression can be recognized or predicted quicker than the actual clinical endpoint using the SEP.[25] Second, the relationship between the surrogate and the clinical endpoint should be established both quantitatively and qualitatively through relevant epidemiological and clinical studies. The nature of this relationship should be understood in terms of pathophysiology or in terms of an expression of joint risk.[25] Lastly, a surrogate endpoint should produce parallel estimates of risks and benefits as the clinical endpoints. The endpoints selected by the authors in the last 10 years all seemingly comply to the first criterion as they describe alterations either in hepatic or systemic parameters. However, we were not able to verify if the other criteria were met, challenging the validity of the obtained results in the studies included in this review. The complexity of validation is perhaps clearly illustrated by two elegant studies which attempted to validate surrogates for mortality following postresectional liver failure.[29,30] Balzan et al. prospectively evaluated 704 patients undergoing hepatic resection.[29] They were able to show that the 50-50 criteria (PT <50% and serum total bilirubin >50 mmol/l on day 5 postoperatively) were an accurate predictor of liver failure and death after a hepatectomy. However, their findings were soon contested by Mullen et al.[30] who conducted a similar study in 1,059 patients undergoing major hepatectomy in which the 50-50 criteria could not be reproduced. The authors therefore introduced a new criterion (peak bilirubin >7.0 mg/dl) that should be considered as a more reliable marker predicting postresectional liver failure and mortality.

Several other medical fields have been trying to standardize the outcomes that are used in clinical trials. Recently, the National Institutes of Health (NIH) and the Agency for Healthcare Research and Quality in the United Kingdom convened an expert group to

propose which biomarkers should be assessed as standardized asthma outcomes in future clinical research studies.[31-33] The challenging task of validating SEPs in liver surgery related trials should follow a similar design and start by assessing and standardizing the definitions of the most used SEPs. It could be achieved either by the conduct of a survey among worldwide HPB surgeons or the formation of an expert panel as demonstrated by the NIH. As a result, a common international prospective database with clear definitions of SEPs could be established. This database would allow the conduct of multicentre trials validating the SEPs in liver surgery. Alternatively, a large multi-centre, multi-national prospective study could be designed to validate the potentially most valuable SEPs. As an example, the Medical Research Council (UK) recently funded a prospective validation study of a combination of the SEPs dimethyl-arginine and ischaemia modified albumin (DASIMAR; MRC 08/H0714/8) in decompensated cirrhosis. Moreover, a recently published study presented an international, multicentre, external validation analysis of the utility interval to biochemical failure (IBF) in predicting prostate cancer mortality at the time of biochemical failure.[34] IBF was chosen as prostatic cancer progression defined by prostate specific antigen, otherwise known as biochemical failure (BF), is almost always the earliest sign of recurrent prostate cancer and can predate clinical manifestations of disease by months to years. Earlier, a large study of 221 men who experienced BF after radiotherapy, a shorter time interval between the completion of treatment and BF [i.e., interval to BF (IBF)] had been shown to be related to the development of distant metastasis and prostate cancer mortality.[35] Thereafter the extensive validation study was designed to substantiate IBF as a SEP for identification of the potentially lethal prostate cancer. These studies are solid examples liver surgery researchers can follow.

In an attempt to define and validate SEPs in hepatic surgery trials, caution should be taken in the choice of SEPs considering the different types of hepatic surgery that can be studied and the patient population that is being investigated.

CONCLUSION

The present systematic review showed that many SEPs are used in hepatic surgery related research. Although these endpoints are biologically plausible, there is little evidence on their validity as true surrogates of clinical endpoints. It is important to standardize SEP definitions and validate the SEPs used in liver surgery trials as the safety is steadily increasing making the differences between interventions smaller and therefore leading to enormous sample sizes. Validated SEPs could be reliable surrogates of clinical endpoints.

REFERENCES

1. Jarnagin WR, Gonen M, Fong Y, DeMatteo RP, Ben-Porat L, Little S et al. Improvement in perioperative outcome after hepatic resection: analysis of 1,803 consecutive cases over the past decade. *Ann Surg* 2002; **236**:397-406.
2. Pulitano C, Aldrighetti L, Arru M, Finazzi R, Catena M, Guzzetti E et al. Preoperative methylprednisolone administration maintains coagulation homeostasis in patients undergoing liver resection: importance of inflammatory cytokine modulation. *Shock* 2007; **28**:401-405.
3. Prescott RJ, Counsell CE, Gillespie WJ, Grant AM, Russell IT, Kiauka S et al. Factors that limit the quality, number and progress of randomized controlled trials. *Health Technol Assess* 1999; **3**:1-143.
4. Ergina PL, Cook JA, Blazeby JM, Boutron I, Clavien PA, Reeves BC et al. Challenges in evaluating surgical innovation. *Lancet* 2009; **374**:1097-1104.
5. Jacquier I, Boutron I, Moher D, Roy C, Ravaud P. The reporting of randomized clinical trials using a surgical intervention is in need of immediate improvement: a systematic review. *Ann Surg* 2006; **244**:677-683.
6. Solomon MJ, McLeod RS. Clinical studies in surgical journals–have we improved? *Dis Colon Rectum* 1993; **36**:43–48.
7. Zwarenstein M, Treweek S, Gagnier JJ, Altman DG, Tunis S, Haynes B et al. Improving the reporting of pragmatic trials: an extension of the CONSORT statement. *BMJ* 2008; **337**:2390.
8. van den Broek MA, van Dam RM, Malago M, Dejong CH, van Breukelen GJ, Olde Damink SW. Feasibility of randomized controlled trials in liver surgery using surgery-related mortality or morbidity as endpoint. *Br J Surg* 2009; **96**:1005-1014.
9. Ellenberg SS. Surrogate endpoints. *Br J Cancer* 1993; **68**:457-459.
10. Buyse M, Molenberghs G, Burzykowski T, Renard D, Geys H. The validation of surrogate endpoints in meta-analyses of randomized experiments. *Biostatistics* 2000; **1**:49-67.
11. Molenberghs G, Geys H, Buyse M. Evaluation of surrogate endpoints in randomized experiments with mixed discrete and continuous outcomes. *Stat Med* 2001; **20**:3023-3038.
12. Prentice RL. Surrogate endpoints in clinical trials: definition and operational criteria. *Stat Med* 1989; **8**:431-440.
13. Fleming TR, DeMets DL. Surrogate end points in clinical trials: are we being misled? *Ann Intern Med* 1996; **125**:605–613.
14. Gluud C, Brok J, Gong Y, Koretz RL. Hepatology may have problems with putative surrogate outcome measures. *J Hepatol* 2007; **46**:734-742.
15. Boissel JP, Collet JP, Moleur P, Haugh M. Surrogate endpoints: a basis for a rational approach. *Eur J Clin Pharmacol* 1992; **43**:235-244.
16. Kaido T, Uemoto S. Quality assessment of randomized controlled trials involving liver surgery. *Hepatogastroenterology* 2008; **55**:1678-1680.
17. Sharma R, Gibbs JF. Recent advances in the management of primary hepatic tumors refinement of surgical techniques and effect on outcome. *J Surg Oncol* 2010; **101**:745-754.
18. Kamel SI, de Jong MC, Schulick RD, Diaz-Montes TP, Wolfgang CL, Hirose K et al. The role of liver-directed surgery in patients with hepatic metastasis from a gynecologic primary carcinoma. *World J Surg* 2011; **35**:1345-1354.
19. Pulitano C, Bodingbauer M, Aldrighetti L, de Jong MC, Castillo F, Schulick RD et al. Liver resection for colorectal metastases in presence of extrahepatic disease: results from an international multi-institutional analysis. *Ann Surg Oncol* 2011; **18**:1380-1388.

20. de Jong MC, Mayo SC, Pulitano C, Lanella S, Ribero D, Strub J et al. Repeat curative intent liver surgery is safe and effective for recurrent colorectal liver metastasis: results from an international multi-institutional analysis. *J Gastrointest Surg* 2009; **13**:2141-2151.
21. van den Broek MA, van Dam RM, van Breukelen GJ, Bemelmans MH, Oussoultzoglou E, Pessaux P et al. Development of a composite endpoint for randomized controlled trials in liver surgery. *Br J Surg* 2011; **98**:1138-1145.
22. Ellenberg S, Hamilton JM. Surrogate endpoints in clinical trials: cancer. *Stat Med* 1989; **8**:405-413.
23. Fleming TR. Surrogate markers in AIDS and cancer trials. *Stat Med* 1994; **13**:1423–1435.
24. Freemantle N, Calvert M. Composite and surrogate outcomes in randomised controlled trials. *BMJ* 2007; **334**:756-757.
25. Espeland MA, O'Leary DH, Terry JG, Morgan T, Evans G, Mudra H. Carotid intimal-media thickness as a surrogate for cardiovascular disease events in trials of HMG-CoA reductase inhibitors. *Curr Control Trials Cardiovasc Med* 2005; **6**:3.
26. Ellenberg SS. Surrogate endpoints: the debate goes on. *Pharmacoepidemiol Drug Saf* 2011; **10**:493-496.
27. Alonso A, Molenberghs G, Geys H, Buyse M, Vangeneugden T. A unifying approach for surrogate marker validation based on Prentice's criteria. *Stat Med* 2006; **25**:205-221.
28. Freedman LS, Graubard BI, Schatzkin A. Statistical validation of intermediate endpoints for chronic diseases. *Stat Med* 1992; **11**:167-178.
29. Balzan S, Belghiti J, Farges O, Ogata S, Sauvanet A, Delefosse D et al. The '50-50 criteria' on post-operative day 5: an accurate predictor of liver failure and death after hepatectomy. *Ann Surg* 2005; **242**:824-828.
30. Mullen JT, Ribero D, Reddy SK, Donadon M, Zorzi D, Gautam S et al. Hepatic insufficiency and mortality in 1,059 noncirrhotic patients undergoing major hepatectomy. *J Am Coll Surg* 2007; **204**:854-862.
31. Busse WW, Morgan WJ, Taggart V, Togias A. Asthma outcomes workshop: overview. J Allergy *Clin Immunol* 2012; **129**.
32. Szefler SJ, Wenzel S, Brown R, Erzurum SC, Fahy JV, Hamilton RG et al. Asthma outcomes: biomarkers. *J Allergy Clin Immunol* 2012; **129**.
33. Cloutier MM, Schatz M, Castro M, Clark N, Kelly HW, Mangione-Smith R et al. Asthma outcomes: composite scores of asthma control. *J Allergy Clin Immunol* 2012; **129**.
34. Buyyounouski MK, Pickles T, Kestin LL, Allison R, Williams SG. Validating the interval to biochemical failure for the identification of potentially lethal prostate cancer. *J Clin Oncol* 2012; **30**:1857-1863.
35. Buyyounouski MK, Hanlon AL, Horwitz EM, Pollack A. Interval to biochemical failure highly prognostic for distant metastasis and prostate cancer-specific mortality after radiotherapy. *Int J Radiat Oncol Biol Phys* 2008; **70**:59-66.
36. Taura P, Fuster J, Mercadal J, Martinez-Palli G, Fondevila C, Blasi A et al. The use of beta-adrenergic drugs improves hepatic oxygen metabolism in cirrhotic patients undergoing liver resection. *J Hepatol* 2010; **52**:340-347.
37. Pietsch UC, Herrmann ML, Uhlmann D, Busch T, Hokema F, Kaisers UX et al. Blood lactate and pyruvate levels in the perioperative period of liver resection with Pringle maneuver. *Clin Hemorheol Microcirc* 2010; **44**:269-281.
38. Domart MC, Esposti DD, Sebagh M, Olaya N, Harper F, Pierron G et al. Concurrent induction of necrosis, apoptosis, and autophagy in ischemic preconditioned human livers formerly treated by chemotherapy. *J Hepatol* 2009; **51**:881-889.

39. Xia F, Wang S, Chen M, Wang X, Feng X, Dong J. Protective effect of Verapamil on hepatic ischemia-reperfusion injury during hepatectomy in the cirrhotic patients with hepatocellular carcinoma. *Langenbecks Arch Surg* 2009; **394**:1041-1046.
40. de Liguori Carino N, O'Reilly DA, Dajani K, Ghaneh P, Poston GJ, Wu AV. Perioperative use of the LiMON method of indocyanine green elimination measurement for the prediction and early detection of posthepatectomy liver failure. *Eur J Surg Oncol* 2009; **35**:957-962.
41. Arkadopoulos N, Kostopanagiotou G, Theodoraki K, Farantos C, Theodosopoulos T, Stafyla V et al. Ischemic preconditioning confers antiapoptotic protection during major hepatectomies performed under combined inflow and outflow exclusion of the liver. A randomized clinical trial. *World J Surg* 2009; **33**:1909-1915.
42. Wen Y, Miao X, Xiong L, Xiong G, Hu J, Zhong D et al. Application of hemihepatic vascular occlusion with hanging maneuver in hepatectomy. *Hepatogastroenterology* 2009; **56**:442-446.
43. Beck-Schimmer B, Breitenstein S, Urech S, De Conno E, Wittlinger M, Puhan M et al. A randomized controlled trial on pharmacological preconditioning in liver surgery using a volatile anesthetic. *Ann Surg* 2008; **248**:909-918.
44. Akita H, Sasaki Y, Yamada T, Gotoh K, Ohigashi H, Eguchi H et al. Real-time intraoperative assessment of residual liver functional reserve using pulse dye densitometry. *World J Surg* 2008; **32**:2668-2674.
45. Ko JS, Gwak MS, Choi SJ, Kim GS, Kim JA, Yang M et al. The effects of desflurane and propofol-remifentanil on postoperative hepatic and renal functions after right hepatectomy in liver donors. *Liver Transpl* 2008; **14**:1150-1158.
46. Scheingraber S, Richter S, Igna D, Flesch S, Kopp B, Schilling MK. Indocyanine green disappearance rate is the most useful marker for liver resection. *Hepatogastroenterology* 2008; **55**:1394-1399.
47. Kim YI, Fujita S, Hwang YJ, Chun JM, Song KE, Chun BY. Successful intermittent application of the Pringle maneuver for 30 minutes during human hepatectomy: a clinical randomized study with use of a protease inhibitor. *Hepatogastroenterology* 2007; **54**:2055-2060.
48. Tang LJ, Tian FZ, Gao XM. Hepatocellular glycogen in alleviation of liver ischemia-reperfusion injury. *Hepatobiliary Pancreat Dis Int* 2002; **1**:532-535.
49. Tang L, Tian F, Tao W, Cui J. Hepatocellular glycogen in alleviation of liver ischemia-reperfusion injury during partial hepatectomy. *World J Surg* 2007; **31**:2039-2043.
50. Brooks AJ, Hammond JS, Girling K, Beckingham IJ. The effect of hepatic vascular inflow occlusion on liver tissue pH, carbon dioxide, and oxygen partial pressures: defining the optimal clamp/release regime for intermittent portal clamping. *J Surg Res* 2007; **141**:247-251.
51. Kostopanagiotou G, Pandazi A, Matsota P, Arkadopoulos N, Dalamanga N, Politou M et al. Effect of packed red blood cells transfusion on plasma fibronectin during liver resection. *Transfus Med* 2007; **17**:115-118.
52. Schmidt SC, Hamann S, Langrehr JM, Hoflich C, Mittler J, Jacob D et al. Preoperative high-dose steroid administration attenuates the surgical stress response following liver resection: results of a prospective randomized study. *J Hepatobiliary Pancreat Surg* 2007; **14**:484-492.
53. Petrowsky H, McCormack L, Trujillo M, Selzner M, Jochum W, Clavien PA. A prospective, randomized, controlled trial comparing intermittent portal triad clamping versus ischemic preconditioning with continuous clamping for major liver resection. *Ann Surg* 2006; **244**:921-928.
54. Kostopanagiotou G, Pandazi AK, Andreadou I, Markantonis SL, Niokou D, Teloudis A et al. Effects of mannitol in the prevention of lipid peroxidation during liver resection with hepatic vascular exclusion. *J Clin Anesth* 2006; **18**:570-574.

55. Theodoraki K, Arkadopoulos N, Fragulidis G, Voros D, Karapanos K, Markatou M et al. Transhepatic lactate gradient in relation to liver ischemia/reperfusion injury during major hepatectomies. *Liver Transpl* 2006; **12**:1825-1831.
56. Dunschede F, Erbes K, Kircher A, Westermann S, Seifert J, Schad A et al. Reduction of ischemia reperfusion injury after liver resection and hepatic inflow occlusion by alpha-lipoic acid in humans. *World J Gastroenterol* 2006; **12**:6812-6817.
57. Esaki M, Sano T, Shimada K, Sakamoto Y, Takahashi Y, Wakai K et al. Randomized clinical trial of hepatectomy using intermittent pedicle occlusion with ischaemic intervals of 15 versus 30 minutes. *Br J Surg* 2006; **93**:944-951.
58. Azoulay D, Lucidi V, Andreani P, Maggi U, Sebagh M, Ichai P et al. Ischemic preconditioning for major liver resection under vascular exclusion of the liver preserving the caval flow: a randomized prospective study. *J Am Coll Surg* 2006; **202**:203-211.
59. Kim YI, Chung HJ, Song KE, Hwang YJ, Lee JW, Lee YJ et al. Evaluation of a protease inhibitor in the prevention of ischemia and reperfusion injury in hepatectomy under intermittent Pringle maneuver. *Am J Surg* 2006; **191**:72-76.
60. Sugimoto H, Okochi O, Hirota M, Kanazumi N, Nomoto S, Inoue S et al. Early detection of liver failure after hepatectomy by indocyanine green elimination rate measured by pulse dye-densitometry. *J Hepatobiliary Pancreat Surg* 2006; **13**:543-548.
61. Figueras J, Llado L, Ruiz D, Ramos E, Busquets J, Rafecas A et al. Complete versus selective portal triad clamping for minor liver resections: a prospective randomized trial. *Ann Surg* 2005; **241**:582–590.
62. Chouker A, Martignoni A, Schauer RJ, Dugas M, Schachtner T, Kaufmann I et al. Alpha-gluthathione S-transferase as an early marker of hepatic ischemia/reperfusion injury after liver resection. *World J Surg* 2005; **29**:528-534.
63. Chouker A, Martignoni A, Schauer R, Dugas M, Rau HG, Jauch KW et al. Beneficial effects of ischemic preconditioning in patients undergoing hepatectomy: the role of neutrophils. *Arch Surg* 2005; **140**:129-136.
64. Kaiho T, Tanaka T, Tsuchiya S, Yanagisawa S, Takeuchi O, Miura M et al. Effect of the herbal medicine Dai-kenchu-to for serum ammonia in hepatectomized patients. *Hepatogastroenterology* 2005; **52**:161-165.
65. Bartels M, Biesalski HK, Engelhart K, Sendlhofer G, Rehak P, Nagel E. Pilot study on the effect of parenteral vitamin E on ischemia and reperfusion induced liver injury: a double blind, randomized, placebocontrolled trial. *Clin Nutr* 2004; **23**:1360-1370.
66. Li SQ, Liang LJ, Huang JF, Li Z. Ischemic preconditioning protects liver from hepatectomy under hepatic inflow occlusion for hepatocellular carcinoma patients with cirrhosis. *World J Gastroenterol* 2004; **10**:2580-2584.
67. Nishiyama T, Fujimoto T, Hanaoka K. A comparison of liver function after hepatectomy in cirrhotic patients between sevoflurane and isoflurane in anesthesia with nitrous oxide and epidural block. *Anesth Analg* 2004; **98**:990-993.
68. Nuzzo G, Giuliante F, Vellone M, De Cosmo G, Ardito F, Murazio M et al. Pedicle clamping with ischemic preconditioning in liver resection. Liver Transpl 2004; **10** (Suppl. 1):S53-S57.
69. Clavien PA, Selzner M, Rudiger HA, Graf R, Kadry Z, Rousson V et al. A prospective randomized study in 100 consecutive patients undergoing major liver resection with versus without ischemic preconditioning. *Ann Surg* 2003; **238**:843-850.
70. Smyrniotis V, Kostopanagiotou G, Lolis E, Theodoraki K, Farantos C, Andreadou I et al. Effects of hepatovenous back flow on ischemicreperfusion injuries in liver resections with the pringle maneuver. *J Am Coll Surg* 2003; **197**:949-954.

71. Wu FP, Boelens PG, van Leeuwen PA, Hoekman K, Hansma AH, Wiezer MJ et al. Effects of major liver resection, with or without recombinant bactericidal/permeability-increasing protein (rBPI21), on the angiogenic profile of patients with metastatic colorectal carcinoma. *J Surg Oncol* 2003; **84**:137-142.
72. Capussotti L, Nuzzo G, Polastri R, Giuliante F, Muratore A, Giovannini I. Continuous versus intermittent portal triad clamping during hepatectomy in cirrhosis. Results of a prospective, randomized clinical trial. *Hepatogastroenterology* 2003; **50**:1073-1077.
73. Man K, Lo CM, Liu CL, Zhang ZW, Lee TK, Ng IO et al. Effects of the intermittent Pringle manoeuvre on hepatic gene expression and ultrastructure in a randomized clinical study. *Br J Surg* 2003; **90**:183-189.
74. Muratore A, Ribero D, Ferrero A, Bergero R, Capussotti L. Prospective randomized study of steroids in the prevention of ischaemic injury during hepatic resection with pedicle clamping. *Br J Surg* 2003; **90**:17-22.
75. Vriens MR, Marinelli A, Harinck HI, Zwinderman KH, van de Velde CJ. The role of allopurinol in human liver ischemia/reperfusion injury: a prospective randomized clinical trial. *Hepatogastroenterology* 2002; **49**:1069-1073.
76. Kim YI, Hwang YJ, Song KE, Yun YK, Lee JW, Chun BY. Hepatocyte protection by a protease inhibitor against ischemia/reperfusion injury of human liver. *J Am Coll Surg* 2002; **195**:41-50.
77. Okochi O, Kaneko T, Sugimoto H, Inoue S, Takeda S, Nakao A. ICG pulse spectrophotometry for perioperative liver function in hepatectomy. *J Surg Res* 2002; **103**:109-113.
78. Wiezer MJ, Boelens PG, Vuylsteke RJ, Nijveldt RJ, Meijer C, Cuesta MA et al. Perioperative treatment with bactericidal/permeabilityincreasing protein (rBPI21) in major liver surgery: a concise summary. *Ann Acad Med Singapore* 2001; **30**:226-233.
79. Orii R, Sugawara Y, Hayashida M, Uchida K, Yamada Y, Takayama T et al. Lactate is correlated with the indocyanine green elimination rate in liver resection for cirrhotic patients. *Anesth Analg* 2001; **92**:1064-1070.
80. Noie T, Sugawara Y, Harihara Y, Takayama T, Kubota K, Ohashi Y et al. Kinetics of urinary trypsin inhibitor in patients undergoing partial hepatectomy. *Scand J Gastroenterol* 2001; **36**:410-416.
81. Yamashita Y, Shimada M, Hamatsu T, Rikimaru T, Tanaka S, Shirabe K et al. Effects of preoperative steroid administration on surgical stress in hepatic resection: prospective randomized trial. *Arch Surg* 2001; **136**:328-333.

Chapter 6

Validation of the peak bilirubin criterion for outcome after partial hepatectomy

Kim M.C. van Mierlo, Toine M. Lodewick, Dipok K. Dhar, Victor van Woerden, Ralph Kurstjens, Frank G. Schaap, Ronald M. van Dam, Soumil Vyas, Massimo Malagó, Cornelis H.C. Dejong, Steven W.M. Olde Damink

HPB (Oxford) 2016 Oct;18(10):806-812

ABSTRACT

Introduction. Postoperative liver failure (PLF) is a dreaded complication after partial hepatectomy. The peak bilirubin criterion (>7.0 mg/dL or ≥120 µmol/L) is often used to define PLF. This study aimed to validate the peak bilirubin criterion as a risk indicator for liver-related mortality within 90 days after partial hepatectomy.

Methods. Patient and surgical characteristics of 956 consecutive patients who underwent partial hepatectomy at the Maastricht University Medical Centre (the Netherlands) or the Royal Free Hospital (United Kingdom) between January 2005 and December 2012 were analysed by uni- and multivariable analyses with odds ratios (OR) and 95% confidence intervals (95%CI).

Results. Thirty-five patients (3.7%) met the postoperative peak bilirubin criterion at median day 19 with a median bilirubin level of 183 [121-588] µmol/L. Sensitivity and specificity for liver-related mortality after major hepatectomy were 41.2% and 94.6%, respectively. The positive predictive value was 22.6%. Independent predictors of liver-related mortality were the peak bilirubin criterion (p<0.001, OR=15.9 [95%CI 5.2-48.7]), moderate-severe steatosis and fibrosis (p=0.013, OR=8.5 [95%CI 1.6-46.6]), ASA 3-4 (p=0.047, OR=3.0 [95%CI 1.0-8.8]) and age (p=0.044, OR=1.1 [95%CI 1.0-1.1]).

Conclusion. The peak bilirubin criterion has a low sensitivity and positive predictive value for 90-day liver-related mortality after major hepatectomy.

INTRODUCTION

Postoperative liver failure (PLF) is a serious complication after partial hepatectomy for benign or malignant liver disease.[1] PLF occurs in approximately 5% of all patients undergoing partial liver resection.[2,3] A liver remnant volume of 25% is regarded as the minimum to prevent PLF.[2] In patients with compromised liver function, up to 40% needs to be preserved.[1]

PLF is characterized by an impaired synthetic, secretory, and detoxifying function of the liver, and accounts for most of the mortality after extensive partial hepatectomy.[1] Strategies to avoid an insufficient remnant liver include staged resection (two-stage hepatectomy,[4] associating liver partition and portal vein ligation for staged hepatectomy (ALPPS)),[5] and portal vein embolization (PVE).[6] Postoperative treatments lack, and only intensive support can be provided when PLF occurs. It is therefore essential to have an accurate postoperative clinical risk indicator that can predict PLF to provide early optimal support.

Three postoperative clinical risk indices that are currently used are the 50-50 criteria (prothrombin time <50% and serum bilirubin >50 µmol/L on postoperative day 5),[7,8] the definition of PLF by the International Study Group of Liver Surgery,[9] and the Model for End-stage Liver Disease.[10,11] However, these parameters have revealed to be suboptimal to detect patients with developing PLF based on their definitions as shown by recent validation studies.[12-14]

In 2007, Mullen et al. proposed a definition for PLF based on analysis of 1,059 patients without cirrhosis who underwent major hepatectomy between 1995 and 2005 at three hepatobiliary centres in the United States and Italy.[15] The authors stated that the occurrence of a systemic bilirubin level of >7.0 mg/dL (≥120 µmol/L, '*peak bilirubin* criterion') within 90 days after major hepatectomy provides a sensitivity of 93.3% for liver-related death and an odds ratio (OR) of 250 (95% confidence interval, 25.0 to >1000) for 90-day liver-related mortality.

Recently, this risk indicator has been retrospectively validated in a prospectively constructed single centre European database.[14] Analysis of 680 hepatectomies in patients without cirrhosis resulted in a positive predictive value of 61.4% for major morbidity and 40.5% for overall mortality when the *peak bilirubin* criterion was applied within 10 days after hepatectomy.[14] However, this is a rather short period of time since the majority of complications occur within 90 days after liver resection.[16]

The *peak bilirubin* criterion is one of the main predictors used in daily practice,[17] but modern practice has changed in the last decade. With the increasing incidence of non-alcoholic fatty liver disease, extending indications for resection,[18] and complex vascular procedures,[19] postoperative morbidity and mortality are negatively affected.[20,21] In addition, chemotherapy-associated liver injury (e.g. sinusoidal obstruction syndrome, steatohepatitis) seems to increase liver-related morbidity and 90-day mortality after liver resection.[21,22]

We hypothesized that following current practice, the criterion would be met more often, and mortality rates would be higher. Therefore, this study aimed to validate the *peak bilirubin* criterion as a postoperative clinical risk indicator regarding major morbidity and liver-related death within 90 days after partial hepatectomy in two European tertiary hepatobiliary referral centres.

METHODS

Patients

Prospectively collected data from all consecutive patients who had undergone minor or major hepatic resection between January 2005 and December 2012 at the Maastricht University Medical Centre (the Netherlands) or the Royal Free Hospital London (United Kingdom) were reviewed. All patients with age >18 years who underwent liver resection were included, independent of ethnicity, quality of the background liver and preoperative systemic levels of liver-related parameters (e.g., bilirubin, alanine aminotransferase). Before surgery, a complete medical history was taken, and patients underwent physical examination and liver-related blood tests. All patients were discussed at a multidisciplinary meeting to determine optimal medical and surgical treatment. Patients who underwent extended hepatectomy received computed tomography-volumetry prior to surgery. Preoperative PVE was performed in patients with a limited predicted functional liver remnant.

Surgical techniques and perioperative management

All liver resections were classified in accordance with the International Hepato-Pancreato-Biliary Association Brisbane nomenclature.[23] Liver resection was performed as described before.[24] To prevent excessive blood loss, central venous pressure was maintained below 5 cm H_2O during transection. Intermittent Pringle manoeuvre was performed in case of increased bleeding risk. Haemostasis was achieved using bipolar coagulation and argon beam coagulation, sutures, and clips. Intraoperative transfusion of packed red blood cells (RBCs) and fresh frozen plasma (FFP) was performed according to hospital protocols.

Postoperatively, patients were admitted to the overnight recovery room or intensive care unit and transferred to the surgical ward the next day if clinically stable.

Definitions

Liver resections were divided into minor (<3 Couinaud segments) and major (≥3 Couinaud segments) resections[26]. Background steatosis was morphologically quantified by histopathologists using a four-graded scale (0-3) as defined by Kleiner et al.[26] Scores 1-3 were considered to correspond with fat deposition in 5-33%, 33-66% and >66% of hepatocytes. Preoperative chemotherapy consisted of adjuvant chemotherapy for the primary tumour, or neoadjuvant chemotherapy to downsize liver metastases within six months before liver surgery. The number of units of transfusion of RBCs or FFP peri-operatively and within 24 hours after surgery was documented. Liver failure was defined according to the *peak bilirubin* criterion of Mullen et al.[15] Postoperative 90-day morbidity was defined in accordance with the surgical Dindo-Clavien classification,[27] and clinically relevant (major) morbidity was defined as a Dindo-Clavien score of 3 or more. Overall mortality was defined as all cause death within 90 days after liver resection. Ninety-day morbidity and mortality were included irrespective of whether this occurred during first admission, after discharge or during readmission. Mortality was subdivided into overall and liver-related (i.e., liver failure, multi-organ failure including liver failure) mortality for validation analyses. Moreover, the liver surgery-specific composite endpoint (LSSCEP) composed of ascites, postoperative liver failure, bile leakage, intra-abdominal haemorrhage, intra-abdominal abscess, and mortality, was used to assess liver surgery-specific complications.[28] Due to implementation of enhanced recovery after surgery (ERAS) programs in liver surgery,[29] bilirubin levels were determined on postoperative day 1, 3, and if deemed necessary, day 5 or beyond. Medical records of all patients were checked up to 90 days after partial hepatectomy.

Statistical analyses

Statistical analyses were performed using SPSS® version 20 (IBM, Armonk, New York, USA). Data are expressed as median [range] and percentages. Sensitivity, specificity, and predictive values for major postoperative morbidity and 90-day liver-related mortality were computed. Clinicopathologic variables associated with morbidity and mortality were examined using univariable and, where applicable, multivariable analyses using logistic regression. For multivariable analysis, a univariable inclusion criterion of $p \leq 0.15$ was used. Statistical significance was considered at a *p-value*<0.05. Results were depicted in p-values with odds ratio (OR) and 95% confidence interval (95%CI).

RESULTS

Patient characteristics

A total of 956 patients were included in this study (Table 1). Four hundred forty-two patients (45.0%) underwent liver resection at the Maastricht University Medical Centre, whereas 514 patients (55.0%) underwent liver surgery at the Royal Free Hospital. Median age of the patients was 64 [20-88] years. Cardiovascular comorbidity (e.g., hypertension, previous myocardial infarction) was present in 442 patients (46.2%) and diabetes mellitus was present in 118 patients (12.3%). 'Other comorbidity' (228 patients, 23.8%) comprised conditions such as hypothyroidism, a history of deep venous thrombosis and/or pulmonary embolism.

Malignant liver disease was the indication for resection in 841 patients (88.0%), with hepatocellular carcinoma as the most common indication in primary liver disease (61 patients, 6.4%), and colorectal liver metastases in metastatic disease (657 patients, 68.7%). Hepatocellular adenoma and cavernous haemangioma (both 19 patients, 2.0%) were the most common benign indications for hepatic resection. Impaired background liver quality was present in 320 patients (219 patients with moderate to severe steatosis, 77 patients with moderate to severe fibrosis and 24 patients with cirrhosis).

Operative details

A total of 490 patients (51.3%) underwent limited resection and 466 patients (48.7%) underwent resection of 3 or more segments (Table 2). Multi-segmentectomies (303 patients, 31.7%) and wedge resections (288 patients, 30.1%) were the most performed surgical procedures followed by a right hepatectomy in 196 patients (20.5%). A total of 426 patients (44.9%) received neoadjuvant chemotherapy. Median surgery time was 267 [45-1200] minutes and median perioperative blood loss was 600 [20-11600] mL. Perioperative transfusion of RBCs and FFP was carried out in 235 (27.5%) and 90 (10.6%) patients, respectively.

Postoperative details

The median length of hospital stay following partial hepatectomy was 9 [2-167] days (Table 2). Overall complications were present in 453 patients (47.4%) and death occurred in 37 patients (3.9%) within 90 days after surgery. Ninety-day major morbidity was present in 244 patients (25.5%) and liver-related mortality in 23 patients (2.4%). Non-liver-related causes of death were of pulmonary (respiratory insufficiency, pneumonia, pulmonary embolus), renal (renal failure), cardiac (myocardial infarction) and gastroenterological (haemorrhage or ischemia) origin. A total of 194 patients (20.3%) met at least one of the criteria of the liver-surgery specific composite endpoint,[28] with intra-abdominal abscess (77 patients, 8.1%) and bile leakage (70 patients, 7.3%) as most frequent complications.

Table 1. Demographic and clinical characteristics of the cohort

Characteristic	n=956
Age (years)	64 [20-88]
Female	409 (42.8)
Comorbidity	
Cardiovascular	442 (46.2)
Pulmonary	107 (11.2)
Diabetes mellitus	118 (12.3)
Renal	16 (1.7)
Other	228 (23.8)
ASA 3 or 4	148 (15.5)
Indication	
Primary hepatic tumour	123 (12.9)
Hepatocellular carcinoma	61 (6.4)
Cholangiocarcinoma	38 (4.0)
Gallbladder carcinoma	21 (2.2)
Other	3 (0.3)
Metastasis	718 (75.1)
Primary colorectal carcinoma	657 (68.7)
Other primary tumour	61 (6.4)
Benign	115 (12.0)
Histology	
Moderate/severe steatosis	219 (22.9)
Moderate/severe fibrosis	77 (8.1)
Cirrhosis	24 (2.5)

Values in parentheses are percentages, values in square brackets depict range. ASA, American Society of Anesthesiologists

Table 2. Peri -and postoperative details of the cohort

Characteristic	n=956
Procedure	
Right hepatectomy	196 (20.5)
Extended right hepatectomy	62 (6.5)
Left hepatectomy	56 (5.9)
Extended left hepatectomy	22 (2.3)
Central resection	27 (2.8)
Wedge/segmentectomy	288 (30.1)
Multisegmentectomy	303 (31.7)
Surgery type	
Minor (<3 Couinaud segments)	490 (51.3)
Major (≥3 Couinaud segments)	466 (48.7)
Preoperative procedures	

Characteristic	n=956
Portal vein embolization	59 (6.2)
Chemotherapy	426 (44.9)
Previous liver resection	106 (11.1)
Intraoperative details	
Laparoscopy	95 (9.9)
Operation time (minutes)	267 [45-1200]
Intraoperative blood loss (mL)	600 [20-11600]
Vascular clamping	
Patients (number)	214 (25.2)
Clamping time (minutes)	30 [8-75]
Transfusion RBCs (units)	
Patients (number)	235 (27.5)
Units (number)	0 [0-25]
Transfusion FFP (units)	
Patients (number)	90 (10.6)
Units (number)	0 [0-13]
Length of stay	
Initial length of stay in hospital	9 [2-167]
Complication grade	
Complications present	453 (47.4)
Dindo-Clavien grade 1	67 (7.0)
Dindo-Clavien grade 2	142 (14.9)
Dindo-Clavien grade 3	134 (14.0)
Dindo-Clavien grade 4	73 (7.6)
Dindo-Clavien grade 5	37 (3.9)
Liver surgery-specific composite endpoint	
Liver surgery-specific composite endpoint present	194 (20.3)
Ascites	19 (2.0)
Liver failure	32 (3.3)
Bile leak	70 (7.3)
Intra-abdominal haemorrhage	25 (2.6)
Intra-abdominal abscess	77 (8.1)
90-day mortality	37 (3.9)
Liver-related mortality	23 (2.4)
Re-admission	
30-day re-admission	97 (10.1)

Values in parentheses are percentages, values in square brackets depict range. RBCs, packed Red Blood Cells; FFP, Fresh Frozen Plasma

Validation of the peak bilirubin criterion

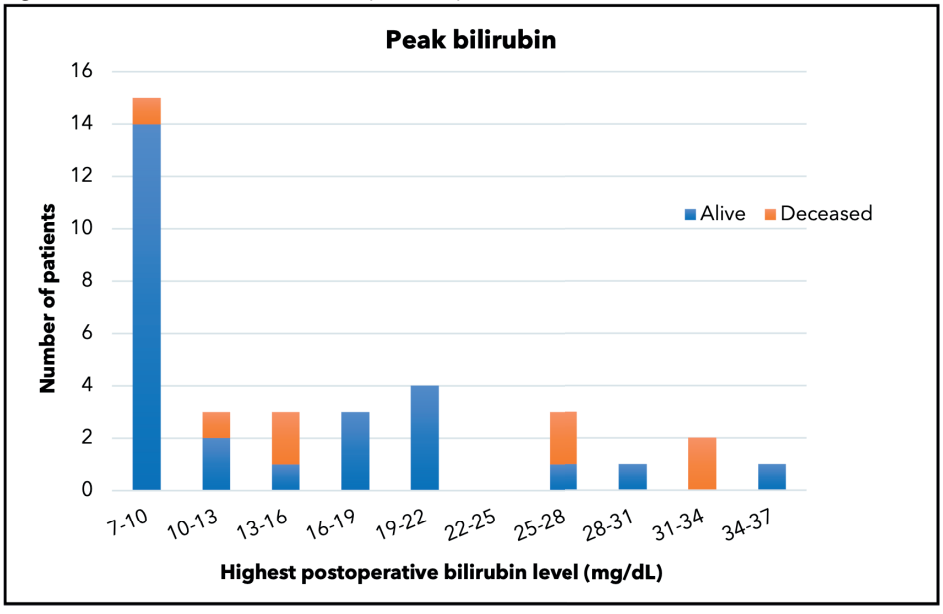
During the postoperative course, the median of the highest bilirubin level measured in all patients was 25 [0-588] µmol/L and occurred on postoperative day 1 [0-53]. The *peak bilirubin* criterion was met in 35 patients (3.7%), with a median bilirubin level of 183 [121-588] µmol/L on postoperative day 19 [5-53]. The positive predictive value of the *peak bilirubin* criterion for liver-related death within 90 days after major liver resection (n=458) was 22.6% (Table 3). Specificity for liver-related death within 90 days was 94.6% after major liver surgery, but sensitivity was only 41.2%. In addition, of all patients with severely elevated bilirubin levels (for the present study defined as >250 µmol/L), 10 patients (66.7%) out of 15 survived and bilirubin levels normalized (Figure 1).

Table 3. Sensitivity, specificity and predictive values of the *peak bilirubin* criterion

	Overall 90-day mortality		Liver-related 90-day mortality	
	All resections (n=936)	Major liver resections (n=459)	All resections (n=934)	Major liver resections (n=458)
Sensitivity	28.6	34.8	36.4	41.2
Specificity	97.2	94.7	97.0	94.6
PPV	28.6	25.8	22.9	22.6
NPV	97.2	96.5	98.4	97.7

PPV, positive predictive value; NPV, negative predictive value

Figure 1. Predictors for liver-related 90-day mortality



In univariable analysis (Table 4), five factors (age, major liver resection, duration of surgery, Pringle manoeuvre, and *peak bilirubin* criterion) were identified as significant prognostic factors affecting liver-related 90-day mortality. Two additional variables (American Society of Anesthesiologists (ASA) 3-4, and the co-occurrence of moderate-severe steatosis and moderate-severe fibrosis/cirrhosis, $p<0.15$) were included in the multivariable analysis. Four independent predictors for liver-related 90-day mortality were identified, with the *peak bilirubin* criterion being the strongest predictor ($p<0.001$, OR=15.9 [95%CI 5.2-48.7]). The other significant predictors for 90-day liver-related mortality were the co-occurrence of moderate-severe steatosis and moderate-severe fibrosis/cirrhosis ($p=0.013$, OR=8.5 [95%CI 1.6-46.6]), ASA 3-4 ($p=0.047$, OR=3.0 [95%CI 1.0-8.8]) and age ($p=0.044$, OR=1.1 [95%CI 1.0-1.1]).

Table 4. Uni –and multivariable analyses on liver-related 90-day mortality (n=956)

Prognostic factor	Univariable		Multivariable	
	***p*-value**	**OR [95%CI]**	***p*-value**	**OR [95%CI]**
Age†	0.002	1.1 [1.0-1.1]	**0.044**	1.1 [1.0-1.1]
Female sex	0.232	0.6 [0.2-1.4]		
ASA 3-4	0.052	2.5 [1.0-6.1]	**0.047**	3.0 [1.0-8.8]
Presence of co-morbidities	0.783	1.1 [0.5-2.7]		
Malignant indication (vs benign)	0.275	3.1 [0.4-23.0]		
Preoperative chemotherapy	0.566	0.8 [0.3-1.8]		
Background liver histology				
Normal	Reference		Reference	
Moderate-severe steatosis	0.499	0.7 [0.2-2.3]	0.754	0.8 [0.2-3.7]
Moderate-severe fibrosis or cirrhosis	0.504	1.7 [0.4-7.5]	0.446	1.9 [0.4-9.8]
Moderate-severe steatosis and fibrosis or cirrhosis	0.116	3.4 [0.7-15.6]	**0.013**	8.5 [1.6-46.6]
Open liver resection (vs. laparoscopic)	0.385	0.4 [0.1-3.1]		
Major liver resection (vs. minor)	0.008	3.9 [1.4-10.6]	0.093	2.9 [0.8-9.7]
Duration of surgery (hours)*	0.026	1.2 [1.0-1.3]		
Blood transfusion*	0.064	2.2 [1.0-5.3]		
Pringle manoeuvre	0.032	2.5 [1.1-6.0]	0.097	2.3 [0.9-5.9]
Peak bilirubin ≥120 µmol/L	<0.001	18.7 [7.3-48.4]	**<0.001**	15.9 [5.2-48.7]

†Age as nominal variable (per year). *Left out due to potential collinearity. OR, odds ratio; CI, confidence interval; ASA, American Society of Anesthesiologists

DISCUSSION

In the present patient cohort, sensitivity, and specificity of the *peak bilirubin* criterion for 90-day liver-related death after major liver resection were 41.2% and 94.6%, respectively,

whereas the positive predictive value only reached 22.6%. In multivariable analysis, the *peak bilirubin* criterion (p<0.001, OR=15.9 [95%CI 5.2-48.7]), co-existing moderate-severe steatosis and moderate-severe fibrosis/cirrhosis (p=0.013), ASA 3-4 (p=0.047), and age (p=0.044) were independent predictors of liver-related death.

The study of Mullen et al. suggests that patients who meet the criterion of the *peak bilirubin* have a more than 30% chance of dying from liver failure after major hepatectomy, in addition to a sensitivity of 93.3% and specificity of 94.3% for liver failure-related mortality. The recent validation study of Skrzypczyk et al. showed a positive predictive value of 40.5% for overall mortality when the *peak bilirubin* criterion was met within 10 days after partial hepatectomy, with a sensitivity and specificity for overall mortality of 56.7% and 96.1%, respectively.

We hypothesized that in the current era of extensive surgeries in patients with (hepatic) comorbidity, more patients would meet the *peak bilirubin* criterion postoperatively and 90-day mortality could be increased due to a lack of treatment. In addition, more patients were expected to meet the criterion when the observation period was 90 days instead of 10 days as in Skrzypczyk et al. Whereas we indeed observed that combined moderate to severe steatosis and severe fibrosis was a significant risk factor for 90-day liver-related mortality after partial hepatectomy, we found a much lower positive predictive value of the *peak bilirubin* criterion. Moreover, of the 15 patients with a severely elevated bilirubin level, 10 patients survived, and bilirubin levels normalized. This confirms the statement of Mullen et al. that the *peak bilirubin* criterion is a turning point instead of a point of no return.[15] In addition, improvements in surgical techniques, preoperative PVE and perioperative care might have led to better support and timely transfer to the intensive care ward.

Even though the present prognostic values were lower than reported by others, the *peak bilirubin* was still identified as conferring the highest risk for 90-day postoperative liver-related mortality in multivariable analysis. Whereas other risk indicators such as the 50-50 criteria or the validation study of Skrzypczyk et al. focus on detection of PLF in the early postoperative course, the present analysis showed that bilirubin levels peaked on median postoperative day 19 indicating that 10 days is too short to detect most patients with this complication.

Although its clinical use is widespread, serum bilirubin reflecting secretory liver function might not be the optimal indicator for poor outcome after partial hepatectomy as shown in a systematic literature review.[30] In the past decades, human and animal studies have shown that plasma and hepatic bile salts are direct indicators of secretory liver function.[31] The relative hepatic overload of bile salts after liver resection[32] in combination with toxicity of

excess hydrophobic bile salts and ensuing impairment of secretory function,[33] can cause a vicious cycle of hepatotoxicity in the remnant liver. Moreover, a delicate intrahepatic balance of bile salts is needed for proper liver regeneration as shown in animal studies.[34] It may be worthwhile to explore the prognostic potential of bile salts in PLF.

Although the clinical characteristics of the present study resemble those of the cohort of Mullen et al., some dissimilarities were present. Whereas having a cirrhotic liver was an exclusion criterion in the latter study, we included patients with cirrhosis (n=24). Furthermore, we included patients with a preoperative serum bilirubin level >2.0 mg/dL (or >34 μmol, n=25), whereas these patients were excluded in Mullen's cohort. Statistical testing, however, did not reveal differences in sensitivity, specificity, and predictive values for the *peak bilirubin* criterion when aforementioned patient groups were excluded (data not shown).

While prospectively collected, data of this study were retrospectively analysed. Due to implementation of enhanced recovery after surgery (ERAS) programs in liver surgery, several systemic parameters such as the international normalized ratio and C-reactive protein were not measured daily.[29,35] Validation of for instance the 50-50 criteria were thus not possible in this cohort.

In conclusion, the present study found a rather low positive predictive value and sensitivity of the *peak bilirubin* criterion for liver-related mortality within 90 days after major liver resection. Nevertheless, it was still identified as the most risk-bearing factor for postoperative liver-related mortality within 90 days after partial liver resection in multivariable analysis. Prospective studies should focus on novel liver function-related parameters such as bile salts, and/or combined functional and volumetric criteria.[36]

REFERENCES

1. van den Broek MA, Olde Damink SW, Dejong CH, Lang H, Malago M, Jalan R et al. Liver failure after partial hepatic resection: definition, pathophysiology, risk factors and treatment. *Liver Int* 2008; **28**:767-780.
2. Schindl MJ, Redhead DN, Fearon KC, Garden OJ, Wigmore SJ, Edinburgh Liver S et al. The value of residual liver volume as a predictor of hepatic dysfunction and infection after major liver resection. *Gut* 2005; **54**:289-296.
3. Okamoto E, Kyo A, Yamanaka N, Tanaka N, Kuwata K. Prediction of the safe limits of hepatectomy by combined volumetric and functional measurements in patients with impaired hepatic function. *Surgery* 1984; **95**:586-592.
4. Lam VW, Laurence JM, Johnston E, Hollands MJ, Pleass HC, Richardson AJ. A systematic review of two-stage hepatectomy in patients with initially unresectable colorectal liver metastases. *HPB* 2013; **15**:483-491.
5. Neumann UP, Dejong CH. (2013) Split decision. *Br J Surg* 2013; **100**:310-312.
6. de Graaf W, van Lienden KP, van den Esschert JW, Bennink RJ, van Gulik TM. Increase in future remnant liver function after preoperative portal vein embolization. *Br J Surg* 2011; **98**:825-834.
7. Balzan S, Belghiti J, Farges O, Ogata S, Sauvanet A, Delefosse D et al. The "50–50 criteria" on postoperative day 5: an accurate predictor of liver failure and death after hepatectomy. *Ann Surg* 2005; **242**:824-828.
8. Paugam-Burtz C, Janny S, Delefosse D, Dahmani S, Dondero F, Mantz J et al. Prospective validation of the "fifty-fifty" criteria as an early and accurate predictor of death after liver resection in intensive care unit patients. *Ann Surg* 2009; **249**:124-128.
9. Rahbari NN, Garden OJ, Padbury R, Brooke-Smith M, Crawford M, Adam R et al. Posthepatectomy liver failure: a definition and grading by the International Study Group of Liver Surgery (ISGLS). *Surgery* 2011; **149**:713-724.
10. Malinchoc M, Kamath PS, Gordon FD, Peine CJ, Rank J, ter Borg PC. A model to predict poor survival in patients undergoing transjugular intrahepatic portosystemic shunts. *Hepatology* 2000; **31**:864-871.
11. Rahbari NN, Reissfelder C, Koch M, Elbers H, Striebel F, Buchler MW et al. The predictive value of postoperative clinical risk scores for outcome after hepatic resection: a validation analysis in 807 patients. *Ann Surg Oncol* 2011; **18**:3640-3649.
12. Schroeder RA, Marroquin CE, Bute BP, Khuri S, Henderson WG, Kuo PC. Predictive indices of morbidity and mortality after liver resection. *Ann Surg* 2006; **243**:373-379.
13. Teh SH, Sheppard BC, Schwartz J, Orloff SL. Model for endstage liver disease score fails to predict perioperative outcome after hepatic resection for hepatocellular carcinoma in patients without cirrhosis. *Am J Surg* 2008; **195**:697-701.
14. Skrzypczyk C, Truant S, Duhamel A, Langlois C, Boleslawski E, Koriche D et al. Relevance of the ISGLS definition of posthepatectomy liver failure in early prediction of poor outcome after liver resection: study on 680 hepatectomies. *Ann Surg* 2014; **260**:865-870.
15. Mullen JT, Ribero D, Reddy SK, Donadon M, Zorzi D, Gautam S et al. Hepatic insufficiency and mortality in 1,059 noncirrhotic patients undergoing major hepatectomy. *J Am Coll Surg* 2007; **204**:854-862.
16. Robertson DJ, Stukel TA, Gottlieb DJ, Sutherland JM, Fisher ES. Survival after hepatic resection of colorectal cancer metastases: a national experience. *Cancer* 2009; **115**:752-759.

17. Egger ME, Ohlendorf JM, Scoggins CR, McMasters KM, Martin, RC, 2nd. Assessment of the reporting of quality and outcome measures in hepatic resections: a call for 90-day reporting in all hepatectomy series. *HPB* 2015; **17**:839-845.
18. van Dam RM, Lodewick TM, van den Broek MA, de Jong MC, Greve JW, Jansen RL et al. Outcomes of extended versus limited indications for patients undergoing a liver resection for colorectal cancer liver metastases. *HPB* 2014; **16**:550-559.
19. Miyazaki M. Combined vascular resection and reconstruction during hepatobiliary and pancreatic cancer surgery. *Br J Surg* 2015; **102**:1-3.
20. Neal CP, Mann CD, Pointen E, McGregor A, Garcea G, Metcalfe MS et al. Influence of hepatic parenchymal histology on outcome following right hepatic trisectionectomy. *J Gastrointest Surg* 2012; **16**:2064-2073.
21. Vauthey JN, Pawlik TM, Ribero D, Wu TT, Zorzi D, Hoff PM et al. Chemotherapy regimen predicts steatohepatitis and an increase in 90-day mortality after surgery for hepatic colorectal metastases. *J Clin Oncol* 2006; **24**:2065-2072.
22. Pessaux P, Chenard MP, Bachellier P, Jaeck D. Consequences of chemotherapy on resection of colorectal liver metastases. *J Visc Surg* 2010; **147**:193-201.
23. Strasberg SM. Nomenclature of hepatic anatomy and resections: a review of the Brisbane 2000 system. *J Hepatobiliary Pancreat Surg* 2005; **12**:351-355.
24. Dejong CHC, Garden OJ. Neoplasms of the liver. In: Majid AA, Kingsnorth A, eds. Advanced surgical practice. London, United Kingdom: Greenwich Medical Media, 2003; pp. 46–56.
25. Couinaud C. Liver anatomy: portal (and suprahepatic) or biliary segmentation. *Dig Surg* 1999; **16**:459-467.
26. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41**:1313-1321.
27. Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. *Ann Surg* 2004; **240**:205-213.
28. van den Broek MA, van Dam RM, van Breukelen GJ, Bemelmans MH, Oussoultzoglou E, Pessaux P et al. Development of a composite endpoint for randomized controlled trials in liver surgery. *Br J Surg* 2011; **98**:1138-1145.
29. Wong-Lun-Hing EM, Lodewick TM, Stoot JH, Bemelmans MH, Olde Damink SW, Dejong CH et al. A survey in the hepatopancreatobiliary community on ways to enhance patient recovery. *HPB* 2012; **14**:818-827.
30. Mpabanzi L, van Mierlo KM, Malago M, Dejong CH, Lytras D, Olde Damink SW. Surrogate endpoints in liver surgery related trials: a systematic review of the literature. *HPB* 2013; **15**:327-336.
31. Esteller A. Physiology of bile secretion. *World J Gastroenterol* 2008; **14**:5641-5649.
32. Jansen PL. Transient elevation of serum bile salts after partial hepatectomy is due to metabolic overload and not to cholestasis. *J Hepatol* 2012; **56**:743-744.
33. Attili AF, Angelico M, Cantafora A, Alvaro D, Capocaccia L. Bile acid-induced liver toxicity: relation to the hydrophobic-hydrophilic balance of bile acids. *Med Hypotheses* 1986; **19**:57-69.
34. Uriarte I, Fernandez-Barrena MG, Monte MJ, Latasa MU, Chang HC, Carotti S et al. Identification of fibroblast growth factor 15 as a novel mediator of liver regeneration and its application in the prevention of post-resection liver failure in mice. *Gut* 2013; **62**:899-910.
35. Hughes MJ, Chong J, Harrison E, Wigmore S. Short-term outcomes after liver resection for malignant and benign disease in the age of ERAS. *HPB* 2016; **18**:177-182.

36. DuBray, BJ, Jr., Levy RV, Balachandran P, Conzen KD, Upadhya GA, Anderson CD et al. Novel three-dimensional imaging technique improves the accuracy of hepatic volumetric assessment. *HPB* 2011; **13**:670-

Chapter 7

Ophthalmic acid as a read-out for hepatic glutathione metabolism in humans

Kim M.C. van Mierlo, Simon A.W.G. Dello, Mechteld C. de Jong, Hans M.H. van Eijk, Theo M. de Kok, Jacob J. Briedé, Frank G. Schaap, Steven W.M. Olde Damink, Cornelius H.C. Dejong

J Clin Transl Res. 2018 Jul 30; 3(Suppl 2): 366–374

ABSTRACT

Background. Animal studies indicated that systemic ophthalmic acid (OPH) is a biomarker for hepatic glutathione (GSH) homeostasis, an important determinant of liver function. We aimed to clarify whether OPH levels can be used as a read-out for hepatic GSH homeostasis after paracetamol (APAP) challenges during pylorus-preserving pancreaticoduodenectomy (PPPD) or partial hepatectomy (PH).

Methods. Nineteen patients undergoing PPPD (n=7, control group) or PH (n=12) were included. APAP (1000 mg) was administered intravenously before resection (first challenge), and six and twelve hours later, with sequential blood sampling during this period. Arterial, hepatic and portal venous blood samples and liver biopsies were taken on three occasions during the first APAP challenge. Plasma and hepatic OPH and GSH levels were quantified, and venous-arterial differences were calculated to study hepatic release.

Results. Systemic GSH levels decreased during the APAP challenge in both surgical groups, without notable change in OPH levels. Hepatic GSH and OPH content was not affected within ~3 hours after administration of the first APAP dose in patients undergoing PPPD or PH. In this period, net release of OPH by the liver was observed only in patients undergoing PPPD.

Conclusion. The drop in circulating GSH levels following APAP administrations, did not result in an increase in plasma OPH in both patients with an intact liver and in those undergoing liver resection. Hepatic content of GSH and OPH was not affected during the first APAP dose. It is uncertain if hepatic GSH homeostasis was sufficiently challenged in the present study (trial number: NL26884.068.09 / 09-3-010).

INTRODUCTION

Prediction of remnant liver function is becoming increasingly important to identify patients at risk of postresectional liver failure.[1-3] One of the important functions of the liver is the defence against diverse forms of (chemical) stress and intoxications.[4-6] For example, radicals are scavenged through reaction with glutathione (GSH), a tripeptide abundantly present in the liver. GSH is synthesized in the cytoplasm, while its degradation by plasma membrane-associated ectoenzymes takes place in the extracellular compartment. The liver releases GSH in bile and sinusoidal blood and is considered to be the predominant source of GSH in the circulation, thus providing extrahepatic tissues with the constituents for local GSH (re)synthesis. Hepatic GSH depletion occurs if the balance between synthesis, intracellular consumption, and release of GSH cannot be maintained, and results in impaired antioxidant defence and attendant cell damage.[7]

One of the processes in which GSH is involved, is the metabolism of the analgesic acetaminophen (APAP). At high doses, a significant fraction of APAP is metabolized by cytochrome P450s giving rise to the reactive compound N-acetyl-p-benzoquinone imine (NAPQI) and phenoxyl radicals derived thereof.[8,9] NAPQI can be neutralized by GSH for subsequent export from the liver. Additionally, GSH reacts with the phenoxyl radical of APAP, resulting in the formation of a less reactive thiyl radical.[10] High doses of APAP may result in a drop in hepatic GSH levels and may cause acute liver failure.[11]

Animal and in vitro studies showed that systemic ophthalmic acid (OPH) levels increased when hepatocellular glutathione and its constituent L-cysteine, were depleted in APAP-induced hepatotoxicity models.[12] OPH is an endogenous tripeptide analogue of GSH and is formed by the same enzymes, with incorporation of 2-aminobutyric acid rather than *L*-cysteine in the initial biosynthetic step.[12-15] OPH lacks a reactive thiol group and is thus devoid of antioxidant properties. It has been suggested that OPH makes use of the same transporter system as GSH and therefore minimizes cellular GSH efflux to preserve cell integrity.[12] Since *L*-cysteine availability is considered the rate-limiting factor in hepatic GSH formation, elevated plasma OPH concentrations may be a read-out of hepatic GSH depletion.

Patients undergoing partial hepatectomy for benign or malignant liver disease often receive APAP pre- and postoperatively to enhance their recovery through reduction of pain.[16] Reduced preoperative liver quality and chemotherapy, as well as extended resections can result in reduced liver function following resection. In the presence of a diminished liver volume and additional surgical stress during partial hepatectomy (PH), the administration of a normal dose of APAP has been suggested to lead to a faster depletion of hepatic

GSH stores.[17] This is one of the reasons why APAP is considered contraindicated after hepatic resection by many clinicians. In the present study we investigated whether an APAP challenge resulted in altered plasma levels and liver content of GSH and OPH in patient groups undergoing abdominal surgery.

MATERIALS AND METHODS

Patient inclusion

All consecutive patients between October 2010 and October 2011 who were older than 18 years and underwent non-laparoscopic liver resection at the Maastricht University Medical Center for malignant disease, were considered for inclusion in this prospective study. In the same time frame, patients undergoing pancreatic surgery were included as a control group with the following rationale: (I) they experience comparable surgical stress as patients undergoing liver resection but their functional liver capacity remains conserved, (II) there are no major differences in anaesthetics during liver and pancreatic surgery, and (III) during pancreatic surgery blood can be drawn more easily from the portal and hepatic vein than during other types of major abdominal surgery allowing the study of splanchnic GSH/OPH release. Exclusion criteria in both groups were alcohol abuse up to six months before participation in this study, aberrations or insufficiency of kidney, liver, gut, heart, or lungs, apart from the underlying malignancy, the presence of persistent inflammation in the gut or liver, the use of drugs known to affect liver metabolism, anaemia or infection, HIV infection, or hepatitis.

Patients were included at the outpatient department and admitted to the hospital one day prior to operation. Routine blood tests were performed at this time. The study was approved by the medical ethical committee of the Maastricht University Medical Center (study number: NL26884.068.09/09-3-010) and conducted according to the revised version of the Declaration of Helsinki (October 2008, Seoul) and the Medical Research Involving Human Subjects Act (WMO). All patients participating in this study gave written informed consent. After surgery, standard clinical care was provided according to the enhanced recovery after surgery (ERAS) protocol.[16]

Outcome and definitions

The primary aim of the study was to investigate whether systemic OPH levels could be an indicator of hepatic GSH depletion. Secondary outcome was the effect of an APAP challenge on generation of thiyl radicals. Liver resections were classified as major (≥3 Couinaud segments) or minor (<3 segments) resections. Morbidity was defined as any

complication within 90 days after surgery, whereas major morbidity comprised complications with a Dindo-Clavien grade of 3 or higher.[18]

Operative procedure

Patients were anaesthetized using isoflurane and propofol. They routinely had an epidural catheter, urinary catheter, two peripheral venous catheters and catheters in a jugular vein and radial artery. Pylorus-preserving pancreaticoduodenectomy (PPPD; modified Whipple procedure) was performed for curation; in case of irresectability, a palliative double bypass was created. In this study, double bypass surgery and PPPD are both referred to as PPPD. PH was performed as detailed elsewhere.[19]

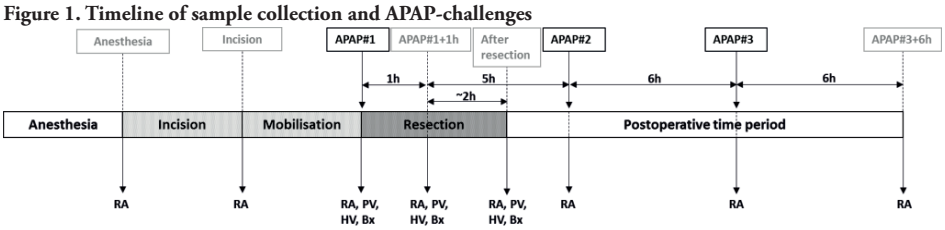
Intravenous APAP-challenge model

APAP (1000 mg, Perfalgan® solution for intravenous infusion, 10 mg/ml, Bristol-Myers Squibb Pharmaceuticals LtD, no organic solvents added) was administered intravenously during surgery directly after mobilization of the liver but before resection (APAP#1), and six (APAP#2) and 12 (APAP#3) hours later (Figure 1). The APAP solution (100ml) was administered in less than five minutes.

Blood and tissue sampling

Radial artery blood samples were obtained at eight predefined time points: after induction of anaesthesia, after incision of the liver/pancreas, immediately before the first APAP challenge (APAP#1), one hour after the first APAP challenge (APAP#1+1h), and after resection of the liver/pancreas (Figure 1). Postoperatively, radial artery blood samples were taken immediately before the second and third APAP challenge (APAP#2 resp. APAP#3), and six hours after the final challenge (APAP#3+6h). For study of venous-arterial differences (ΔVA), blood was drawn near-simultaneously from the radial artery, portal vein, and one of the hepatic veins. This was performed on three occasions, *viz.* at APAP#1, APAP#1+1h, and after completion of liver/pancreatic resection. Concurrent intra-operative liver biopsies were taken in both groups on these time points. In case of liver resection, blood samples and biopsies were taken from the non-tumour bearing hemi-liver.

Figure 1. Timeline of sample collection and APAP-challenges



Transection, transection of the liver or pancreas; APAP, 1000 mg paracetamol intravenously; Bx, liver biopsy; Arterial, blood sample from radial artery, PV, blood sample from portal vein; HV, blood sample from one of the hepatic veins

VA differences (ΔVA) across the portal drained viscera (PDV), liver and splanchnic area (SPL) were calculated using the following formulae:

ΔVA_{PDV} = portal venous [X] – arterial [X]

Liver input = 0.30 * arterial [X] + 0.70 * portal venous [X]

ΔVA_{Liver} = hepatovenous [X] – liver input

ΔVA_{SPL} = hepatovenous [X] – arterial [X]

Sample preparation and OPH/GSH measurements

Blood samples were collected in pre-chilled heparinized vacuum tubes (6 mL) and immediately centrifuged at 4°C at 3500*g for 10 minutes in the operating theatre. Obtained plasma samples were stored at -80°C until analysis. For OPH and GSH measurements, plasma samples were deproteinized by the addition of an equal volume of a freshly prepared 5% (w/v) 5-sulphosalicylic acid solution containing 0.1% (w/v) vitamin C (British Drug Houses, Amsterdam, The Netherlands) to prevent oxidation of GSH. Liver samples were homogenized by microbeating 10 mg of liver tissue in 100 microliters of the above solution, and homogenates were stored at -80°C until analysis. Prior to analysis, all samples were centrifuged for 10 minutes at 50000 *g at 4°C. OPH and GSH were measured in plasma and liver homogenate supernatants using liquid chromatography-mass spectrometry.[20]

Quantification of thiyl radicals

Thiyl radicals were assayed using electron spin resonance (ESR) spectroscopy at low temperature.[21] For this, frozen human liver biopsies were placed in liquid nitrogen in a quartz liquid finger Dewar at the centre of the spectrometer's high sensitivity cavity. ESR spectra were recorded on an X-band spectrometer (Bruker EMX 1273, Biospin, Rheinstetten, Germany) operating at 9.50 GHz. Instrumental settings were magnetic field: 3325 G; scan range: 150 G; modulation frequency 100 kHz modulation amplitude: 5 G; receiver gain: 1 x 10^5; power: 20 mW; time constant: 20.84 ms; scan time: 40.96 ms; number of scans: 20. Thiyl radicals were quantified by peak surface measurements using the WIN-EPR spectrum manipulation program (Version 2.11, Bruker, Rheinstetten, Germany).

Statistical analyses

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS®) version 24.0 (IBM, New York). All data are expressed as median with interquartile range. To compare categorical variables in the two surgical groups the Mann-Whitney *U*

and Fisher's Exact Test were applied where appropriate. Effects of the peri-operative APAP challenge on circulating and hepatic GSH and OPH levels were analysed using the Friedman test for repeated measurements. If appropriate, pre-defined pair-wise comparisons for circulating (baseline (=anaesthesia) *versus* all other time points) and hepatic (baseline (=APAP#1) *versus* all other time points) analytes were made with a posthoc Wilcoxon signed-rank test and Bonferroni-Holm correction for multiple comparisons. Correlations between systemic GSH and OPH were evaluated with Spearman's rank test for non-parametric data. Venous-arterial gradients (ΔVA) of GSH and OPH were tested versus a theoretical median of zero using the Wilcoxon signed-rank test. A p-value <0.05 was considered statistically significant.

RESULTS

Patient characteristics

Nineteen patients (seven females; 12 males) scheduled for a PPPD or liver resection were included in this study (Table 1). Seven patients had a pancreatic malignancy, of whom four underwent a PPPD and three received a palliative double bypass. None of these patients received neoadjuvant chemotherapy. Twelve patients underwent a liver resection for primary (n=2; isolated cases of intrahepatic cholangiocarcinoma and hepatocellular carcinoma) or secondary (n=10, nine cases of CRLM, single case of metastases of a melanoma) liver malignancies. Neoadjuvant chemotherapy was administered in six out of 12 patients undergoing liver resection.

Three patients underwent major liver resection. Biochemical assessment showed no significant differences between the surgical groups in liver-related parameters (Table 1). Histopathological evaluation revealed that none of the patients had cirrhosis of the liver (a condition associated with reduced intrahepatic GSH levels).[22] Regarding the postoperative course, no significant differences were observed between surgical groups regarding length of hospital stay, overall and major morbidity (Table 1).

Effect of APAP challenge on arterial GSH and OPH levels

Plasma levels of GSH and OPH were not different (p=0.536 and p=0.432, respectively) between surgical groups at baseline (i.e., time point of anaesthesia) (Figure 2). A significant change in time was observed for plasma GSH levels in both surgical groups (p=0.001 for both groups). Post-hoc pair wise comparisons of the respective time points versus baseline reached no significance in the PPPD group, whereas GSH levels were significantly lower 2-3 hours after resection of the liver (i.e., APAP#2 time point, p=0.003, Figure 2A). Plasma OPH levels changed significantly over time in patients undergoing PPPD

Table 1. Patient and surgery-related characteristics

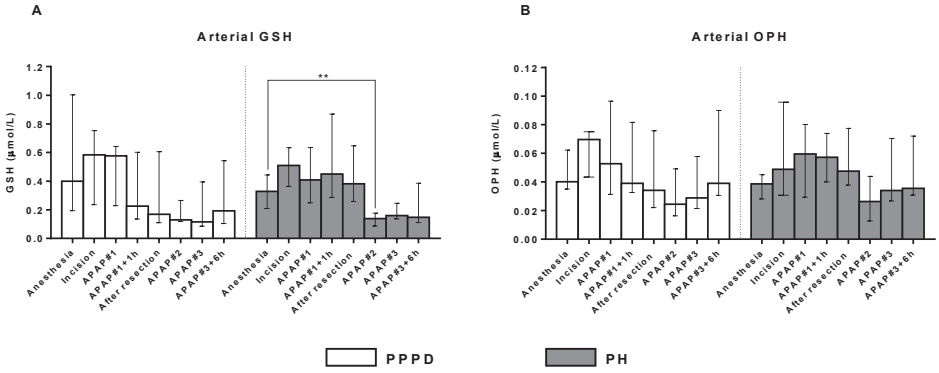
	Pancreatic resection group (PPPD) (n=7)	Partial hepatectomy (PH) (n=12)	*p*-value
Gender			
Male	3	9	1.000§
Female	4	3	
Median age (years)	67 [60-73]	65 [40-71]	0.592†
Median BMI	24.4 [21.1-31.8]	26.5 (22.8-35.8]	0.261†
Preoperative laboratory values			
AST (IU/L)	19 [17-24]	24 [8-85]	0.368†
ALT (IU/L)	34 [24-41]	29 [12-140]	0.659†
LDH (IU/L)	138 [122-154]	197 [125-455]	0.073†
GGT (IU/L)	75 [74-128]	66 [28-308]	0.659†
AP (IU/L)	138 [125-138]	112 [68-522]	0.100†
Bilirubin (micromol/L)	21 [18-45]	13 [6-53]	0.145†
Creatinine (micromol/L)	65 [43-108]	77 [49-108]	0.227†
Preoperative chemotherapy			
No	7	6	0.044§
Yes	0	6	
Indication for surgery			
Primary malignancy	7	2	n/a
Secondary malignancy	0	10	
PVE			
No	7	11	n/a
Yes	0	1	
Pringle maneuver			
No	7	8	n/a
Yes	0	4	
Postoperative short-term outcome			
Median hospital stay	12 [3-57]	9 [5-29]	0.227†
Overall morbidity			
No	1	7	0.147§
Yes	6	5	
Major morbidity (DC III-V)			
No	3	9	0.326
Yes	4	3	
Liver surgery-specific complications			
No	6	10	1.000§
Yes	1	2	

Values depicted in median with range. §Fisher's Exact test, †Mann-Whitney *U* test. PVE, portal vein embolization

(p=0.013) or PH (p=0.005), although the directionality in time was less clear than for GSH (Figure 2B). The latter appeared to be reflected in lack of significant changes in direct pair wise comparisons of time points *versus* baseline. Direct comparisons between the surgical groups revealed that neither GSH nor OPH levels differed significantly at any of the time points during the APAP challenge (data not shown).

Similar directionality of correlations between arterial GSH and OPH was observed upon stratification for type of surgery (data not shown), hence data of all patients were pooled to increase power of correlation analysis. Arterial GSH and OPH were positively correlated at all time points (ρ between 0.51-0.90, supplemental data, Appendix 1).

Figure 2. Ophthalmic acid and glutathione levels in arterial plasma



Time course of **(A)** OPH and **(B)** GSH plasma levels in both groups. Data were plotted as mean and SEM, **p<0.01 versus baseline. GSH, glutathione; OPH, ophthalmic acid; PPPD, pylorus-preserving pancreaticoduodenectomy; PH, partial hepatectomy; APAP, acetaminophen; h, hour

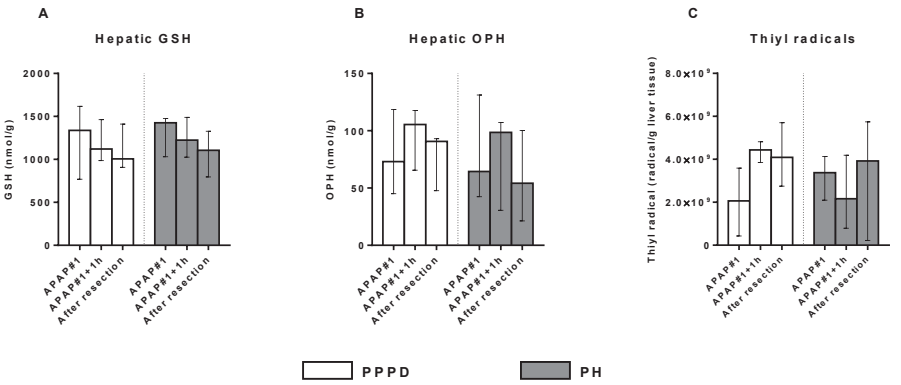
Effect of APAP challenge on hepatic levels of GSH, OPH and APAP thiyl radicals

Hepatic GSH content prior to first APAP administration (APAP#1 time point) was similar in patients undergoing PPPD and PH ((1338 [769-1617] vs. 1425 [1030-1475] nanomol/g liver, resp.; p=0.750) (Figure 3A). The median time between start (APAP#1) and end of resection was 122 [70-215] minutes and did not differ between surgical groups (p=0.145). Hepatic GSH did not change over time in patients undergoing PPPD (p=0.779) or PH (p=0.247).

Baseline hepatic OPH content was not different in patients undergoing PPPD or PH (73 [45-119] vs. 64 [42-131] nanomol/g liver, resp.; p=0.892), and levels did not change during the course of resection in either patients undergoing PPPD (p=0.779) or PH (p=0.247) (Figure 3B).

Likewise, baseline levels of APAP-derived thiyl radicals were similar in liver of patients undergoing PPPD or PH ($2.1{\cdot}10^9$ [$0.36\text{-}5.6{\cdot}10^9$] vs. $3.4{\cdot}10^9$ [$0.11\text{-}6.7{\cdot}10^9$] radicals/g liver, resp.; p=0.335), and levels did not change over time in either group (p=0.717 and p=0.867, resp.) (Figure 3C).

Figure 3. GSH, OPH and thiyl radicals in liver tissue



GSH **(A)**, OPH **(B)**, and thiyl radical **(C)** levels measured at consecutive time points in homogenized liver biopsies of patients undergoing a PPPD or PH. Data were plotted as mean and SEM. PPPD, pylorus-preserving pancreatoduodenectomy; PH, partial hepatectomy; GSH, glutathione; OPH, ophthalmic acid; ESR, electrospin resonancy; APAP, acetaminophen; h, hour

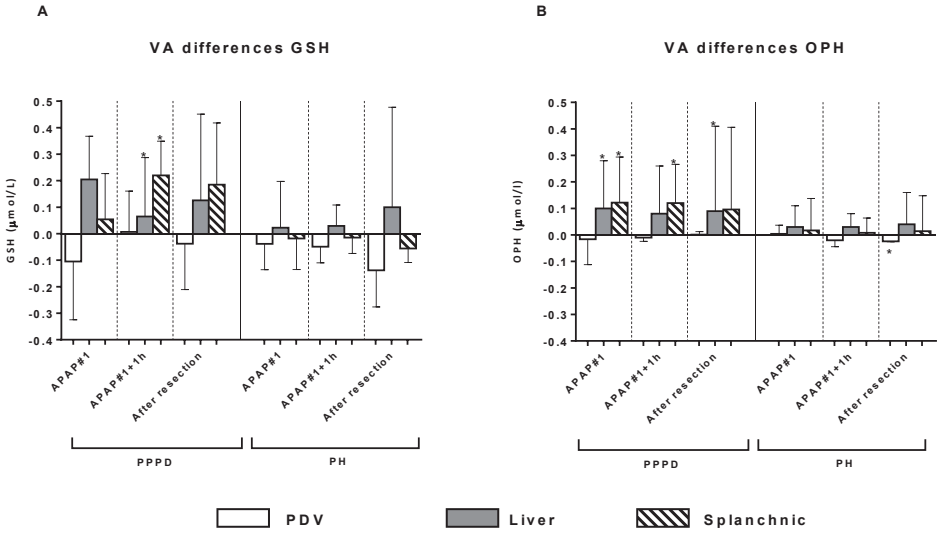
Effect of APAP on hepatic movement of GSH and OPH

Simultaneous drawing of portal venous, radial arterial and hepatic venous blood at three occasions during the first APAP administration, allowed the assessment of the early effects of this challenge on the net extraction or net release of GSH and OPH by the liver (Appendix 2).

Contrary to expectation, there was no net release of GSH from the liver at baseline in patients undergoing PPPD (p=0.297) or PH (p=0.677), although net release was apparent one hour after APAP administration in the PPPD group (p=0.031) (Figure 4A, grey bars). In contrast, net hepatic and splanchnic release of OPH was observed at baseline and both time points after APAP challenge in patients undergoing PPPD (p between 0.016 and a trend of 0.063), but not in patients undergoing liver resection (Figure 4B).

VA differences for GSH across the PDV were not significant at baseline or at later time points in either group, indicating that there was no net movement of GSH across the tissues draining into the portal vein (Figure 4A, white bars). Likewise, in general there was no net movement of OPH across the PDV during the first APAP administration, although net extraction by the PDV did occur after completion of liver resection (p=0.031) (Figure 4B, white bars).

Figure 4. Venous-arterial differences in visceral tissues



Venous-arterial concentration gradients of GSH and OPH across the PDV, liver and splanchnic area in patients undergoing PPPD **(A)** or PH **(B)** tested versus a theoretical median of zero. Data were plotted as median with interquartile range. *p<0.05 versus a theoretical median of zero. PDV; portal drained viscera, PPPD, pylorus-preserving pancreatoduodenectomy; PH, partial hepatectomy; GSH, glutathione; OPH, ophthalmic acid; APAP, acetaminophen; h, hour

DISCUSSION

In the present study we investigated whether plasma OPH is useful as a read-out for hepatic GSH depletion in humans. To this end, a total of three doses of APAP were administered in a peri-operative time frame of 18 hours, to patients with preserved (PPPD group) and reduced (PH group) liver mass. Our main finding is that the decline in plasma GSH, observed in both groups during APAP challenge, was not accompanied by a reciprocal increase in plasma OPH. Rather, the positive correlation between circulating GSH and OPH under a clinically realistic APAP regimen, calls for careful consideration of data from earlier animal and *in vitro* experiments.

Acute effects of the first gift of APAP on hepatic GSH homeostasis could be studied in the ~3-hour interval between start and completion of the respective resection procedures. Within this time frame there were no alterations in hepatic GSH or OPH content, nor was there enhanced production of thiyl radicals in either patient group (Figure 3). Although the liver is considered the predominant source of GSH in the circulation,[23] we did not observe net hepatic GSH release prior to, or after APAP administration in the present study (Figure 4). Net hepatic and splanchnic release of OPH was observed though in patients undergoing PPPD, with a similar magnitude maintained during the 3 hours after

the first APAP administration. Above findings indicate that APAP did not result in acute oxidative stress or prompt alterations in hepatic GSH homeostasis. Obviously, longer term effects of the sequential APAP administrations could not be studied at the level of the liver. The integral APAP challenge resulted in significant lowering of circulating GSH levels (Figure 2). Plasma GSH levels were similar in both surgical groups at all studied time points. Hence, removal of part of the liver, considered the main source of circulating GSH, did not result in a further decline of plasma GSH. The latter is consistent with the absence of net hepatic GSH release in the current study.

Although animal studies revealed elevation of plasma OPH following APAP-induced depletion of hepatic and circulating GSH, an inverse relationship between plasma GSH and OPH was not apparent in our patients. The applied APAP doses in this study were equal to doses used in standard postoperative care and comparable with normal clinical practice (maximum of 4000 milligrams a day). Cumulative APAP dose was rather low in comparison to levels attained in *in vitro* models and mouse studies, the latter with concentrations up to 600 mg/kg body weight.[9,12,13] Geenen et al. used a mathematical model based on data from hepatic cell lines to predict intracellular and extracellular concentrations of OPH following APAP administration.[24] Extracellular OPH concentrations remained stable until the intracellular GSH concentration decreased under a threshold, after which OPH production increased. Based on above studies it can be concluded that OPH is a good marker for hepatic GSH homeostasis under conditions of severe GSH depletion. Unchanged intra-operative hepatic content of GSH, OPH, and APAP-derived thiyl radicals indicate that those conditions were not met in the current human model, at least not in the first three hours after the initial APAP dose. Assuming an average liver weighs 1500g, the current experiment consumed 0.22 µmol GSH/ g liver during the conjugation of NAPQI. Considering hepatic GSH levels of 1.5 µmol GSH/ g liver, about 15% of the hepatic GSH levels are being used. This is far less than the 70->90% of hepatic GSH loss after an overdose in mice. Increasing the APAP dose would not be justified because of concerns of acute liver failure,[25,26] especially for patients undergoing (liver) surgery.

Arterial GSH and OPH were positively correlated at all studied time points. Since OPH and GSH are synthesized through the same enzymatic machinery,[12] is it likely that there is competition between the initial substrates which may explain the same dynamics in plasma. In the present study, hepatic GSH and OPH levels did not correlate with their respective levels in plasma (p between 0.071-1.000). This is in contrast with findings of Soga et al. who showed a good correlation between hepatic and systemic OPH in an APAP-related mouse model,[12] and demonstration by Kombu et al. that 2H labelling of plasma GSH was an indicator for 2H labelling of liver GSH in a rat model.[14] An explanation could be that in the rat model of Kombu et al. arterial blood samples were taken from

the aorta, whereas in the mouse model of Soga et al. it is unclear from what puncture site blood samples were taken. Geenen et al. measured OPH concentrations in medium of cultured hepatic cell lines.[27] All studies thus have potentially assessed OPH concentrations in different fluid compartments, and the influence of this on the results is unclear.

The fact that APAP administration during liver surgery did not lead to (immediate) GSH depletion or increased OPH levels granted valuable information about the safety of administration of APAP used after liver surgery in a standard postoperative care program. Based on stable levels of hepatic GSH, OPH and thiyl radicals during surgery, standard APAP administration seems to be safe in this specific population with regards to GSH homeostasis. However, no general statements can be made on the basis of the current experiment, since GSH homeostasis and susceptibility to xenobiotic toxicity are influenced by numerous factors including genetic polymorphism in glutamate cysteine ligase,[28] altered levels of the expression of genes encoding the γ-glutamyl transpeptidase enzyme, and GSH synthase deficiency and changes in methionine metabolic pathway (i.e. in cirrhotic patients and in patients with homocysteinemia).[29] Even in healthy individuals, peak ALT levels more than 8 times their baseline have been reported in 27% of participants upon APAP administration in therapeutic doses.[30-32] Therefore, caution is still warranted with APAP as a postoperative analgesic following liver resection.

The present study is hampered by some limitations. The APAP solution that was used in this study contained 0.1 mg/mL *L*-cysteine. Although this could have affected GSH/OPH synthesis, this amount is 80 times lower on a molar basis than the amount of APAP administered. In patients suffering from APAP-intoxication, the amount of *L*-cysteine that is repeatedly administered is more than 1000 times higher. In addition, only three patients underwent major hepatectomy. It was therefore impossible to determine the effect of liver resection volume on arterial or hepatic GSH and OPH. At last, the influence of anaesthesia on GSH and OPH metabolism is unknown, and it would be worthwhile to assess arterial OPH and GSH before induction of anaesthesia as optimal baseline measure.

In conclusion, this is the first human study in which the usefulness of OPH as a read-out for hepatic GSH metabolism was explored. APAP administration had no acute effects on hepatic levels of GSH and OPH, and eventually resulted in lowering of GSH in the circulation. Plasma GSH and plasma OPH were positively correlated at all time points during the APAP challenges, and this raises the question whether hepatic GSH homeostasis was sufficiently challenged in the current study. Alternatively, findings from animal studies may be explained by APAP dosing effects. Future studies are needed to examine validity of plasma OPH as a biomarker for hepatic GSH depletion in clinical practice. Informative patient groups may be patients with acute (APAP intoxication) or postresectional liver failure.[33,34]

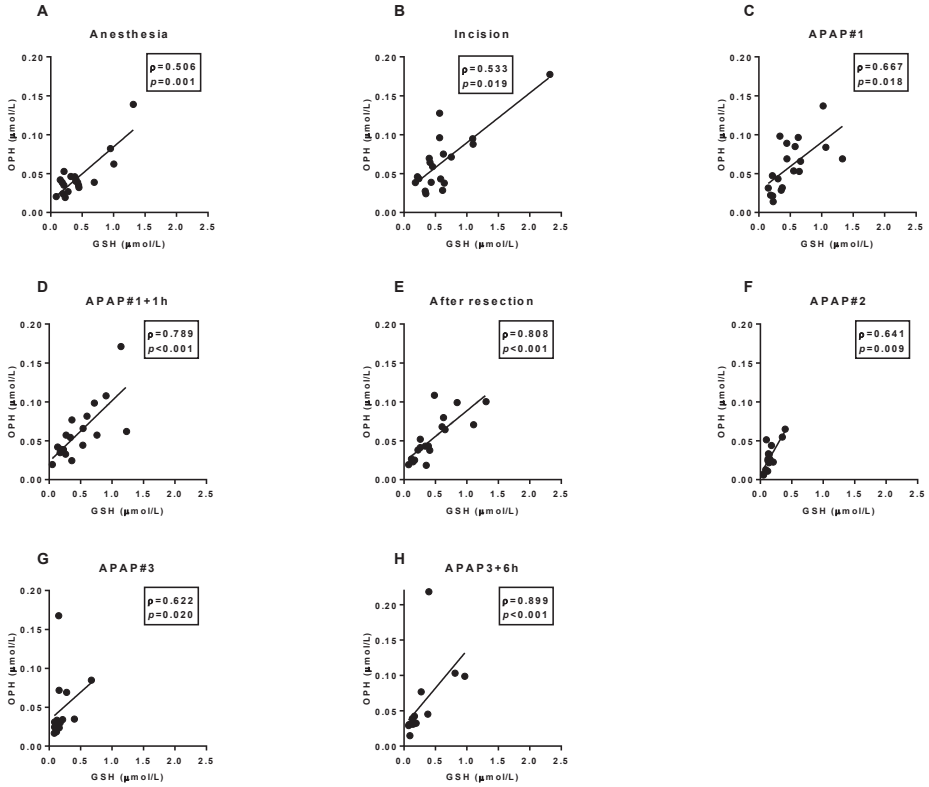
REFERENCES

1. van den Broek MA, Olde Damink SW, Dejong CH, et al. Liver failure after partial hepatic resection: definition, pathophysiology, risk factors and treatment. *Liver Int* 2008; **28**:767-780.
2. Hoekstra LT, de Graaf W, Nibourg GA, et al. Physiological and biochemical basis of clinical liver function tests: a review. *Ann Surg* 2013; **257**:27-36.
3. van Mierlo KM, Schaap FG, Dejong CH, Olde Damink SW. Liver resection for cancer: new developments in prediction, prevention and management of postresectional liver failure. *J Hepatol* 2016; **65**:1217-1231.
4. Hinson JA, Reid AB, McCullough SS, James LP. Acetaminophen-induced hepatotoxicity: role of metabolic activation, reactive oxygen/nitrogen species, and mitochondrial permeability transition. *Drug metabolism reviews* 2004; **36**:805-822.
5. Biolo G, Antonione R, De Cicco M. Glutathione metabolism in sepsis. *Crit Care Med* 2007; **35**:591-595.
6. Hammond CL, Lee TK, Ballatori N. Novel roles for glutathione in gene expression, cell death, and membrane transport of organic solutes. *J Hepatol* 2001; **34**:946-954.
7. Schauer RJ, Gerbes AL, Vonier D, et al. Glutathione protects the rat liver against reperfusion injury after prolonged warm ischemia. *Ann Surg* 2004; **239**:220-231.
8. Jaeschke H, McGill MR, Ramachandran A. Oxidant stress, mitochondria, and cell death mechanisms in drug-induced liver injury: lessons learned from acetaminophen hepatotoxicity. *Drug metabol rev* 2012; **44**:88-106.
9. McGill MR, Lebofsky M, Norris HR, et al. Plasma and liver acetaminophen-protein adduct levels in mice after acetaminophen treatment: dose-response, mechanisms, and clinical implications. *Tox and Appl Pharm* 2013; **269**:240-249.
10. Ramakrishna Rao DN, Fischer V, Mason RP. Glutathione and ascorbate reduction of the acetaminophen radical formed by peroxidase. Detection of the glutathione disulfide radical anion and the ascorbyl radical. *J Biol Chem* 1990; **265**:844-847.
11. Jaeschke H, Williams CD, Ramachandran A, Bajt ML. Acetaminophen hepatotoxicity and repair: the role of sterile inflammation and innate immunity. *Liver Int* 2012; **32**:8-20.
12. Soga T, Baran R, Suematsu M, et al. Differential metabolomics reveals ophthalmic acid as an oxidative stress biomarker indicating hepatic glutathione consumption. *J Biol Chem* 2006; **281**:16768-16776.
13. Abbas R, Kombu RS, Ibarra RA, Goyal KK, Brunengraber H, Sanabria JR. The Dynamics of Glutathione Species and Ophthalmate Concentrations in Plasma from the VX2 Rabbit Model of Secondary Liver Tumors. *HPB Surg* 2011; **2011**:709052.
14. Kombu RS, Zhang GF, Abbas R, et al. Dynamics of glutathione and ophthalmate traced with 2H-enriched body water in rats and humans. *Am J Physiol Endocrinol Metab* 2009; **297**:260-269.
15. Dello SA, Neis EP, de Jong MC, et al. Systematic review of ophthalmate as a novel biomarker of hepatic glutathione depletion. *Clin Nutr* 2012; **32**:325-330.
16. van Dam RM, Hendry PO, Coolsen MM, et al. Initial experience with a multimodal enhanced recovery programme in patients undergoing liver resection. *Br J Surg* 2008; **95**:969-975.
17. Abu-Amara M, Yang SY, Tapuria N, Fuller B, Davidson B, Seifalian A. Liver ischemia/reperfusion injury: processes in inflammatory networks--a review. *Liver Transpl* 2010; **16**:1016-1032.
18. Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. *Ann Surg* 2004; **240**:205-213.
19. Dejong C, Garden O. Neoplasms of the liver. In: Majid AA Kingsnorth A, eds Advanced surgical practice. London: Greenwich medical Media 2003:146-156.

20. Dello SA, van Eijk HM, Neis EP, de Jong MC, Olde Damink SW, Dejong CH. Ophthalmate detection in human plasma with LC-MS-MS. *J Chromatogr B Analyt Technol Biomed Life Sci* 2012; **903**:1-6.
21. Sevilla MD, Becker D, Swarts S, Herrington J. Sulfinyl radical formation from the reaction of cysteine and gluthathione thyil radicals with molecular oxygen. *Biochem and Biophys res Comm* 1987; **144**:1037-1042.
22. Czeczot H, Scibior D, Skrzycki M, Podsiad M. Glutathione and GSH-dependent enzymes in patients with liver cirrhosis and hepatocellular carcinoma. *Acta Biochim Pol* 2006; **53**:237-242.
23. Lauterburg BH, Adams JD, Mitchell JR. Hepatic glutathione homeostasis in the rat: efflux accounts for glutathione turnover. *Hepatology* 1984; **4**:586-590.
24. Geenen S, du Preez FB, Snoep JL, et al. Glutathione metabolism modeling: a mechanism for liver drug-robustness and a new biomarker strategy. *Biochim Biophys Acta* 2013; **1830**:4943-4959.
25. Ostapowicz G, Fontana RJ, Schiodt FV, et al. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. *Ann Intern Med* 2002; **137**:947-954.
26. Craig DG, Lee A, Hayes PC, Simpson KJ. Review article: the current management of acute liver failure. *Aliment Pharmacol Ther* 2010; **31**:345-358.
27. Geenen S, Michopoulos F, Kenna JG, Kolaja KL, Westerhoff HV, Wilson I. HPLC-MS/MS methods for the quantitative analysis of ophthalmic acid in rodent plasma and hepatic cell line culture medium. *J Pharm Biomed Anal* 2011; **54**:1128-1135.
28. Walsh AC, Feulner JA, Reilly A. Evidence for functionally significant polymorphism of human glutamate cysteine ligase catalytic subunit: association with glutathione levels and drug resistance in the National Cancer Institute tumor cell line panel. *Toxicol Sci* 2001; **61**:218-223.
29. Lu SC. Regulation of hepatic glutathione synthesis: current concepts and controversies. *FASEB J* 1999; **13**:1169-1183.
30. Watkins PB, Kaplowitz N, Slattery JT, et al. Aminotransferase elevations in healthy adults receiving 4 grams of acetaminophen daily: a randomized controlled trial. *JAMA* 2006; **296**:87-93.
31. Jalan R, Williams R, Bernuau J. Paracetamol: are therapeutic doses entirely safe? *Lancet* 2006; **368**:2195-2196.
32. Watelet J, Laurent V, Bressenot A, Bronowicki JP, Larrey D, Peyrin-Biroulet L. Toxicity of chronic paracetamol ingestion. *Aliment Pharmacol Ther* 2007; **26**:1543-1544.
33. Donnelly MC, Davidson JS, Martin K, Baird A, Hayes PC, Simpson KJ. Acute liver failure in Scotland: changes in aetiology and outcomes over time (the Scottish Look-Back Study). *Aliment Pharmacol Ther* 2017; **45**:833-843.
34. Kaur et al. Detection of Ophthalmic Acid in Serum from Acetaminophen-Induced Acute Liver Failure Patients Is More Frequent in Non-Survivors. *PLoS One* 2015; **25**:10.

SUPPLEMENTAL DATA

Figure 1. Correlations between arterial GSH and OPH levels



Correlations between arterial GSH and OPH levels at all time points in patients undergoing PPPD and PH **(A-H)**. GSH, glutathione; OPH; ophthalmic acid; APAP, acetaminophen; h, hour; ρ, Spearman's correlation coefficient

Figure 2. Values of venous-arterial differences of GSH and OPH in visceral tissues

GSH VA gradients		PDV	*p*-value	Liver	*p*-value	Splanchnic	*p*-value
APAP#1	PPPD (n=7)	-0.105 [-0.325-0.082]	0.156	0.205 [-0.064-0.368]	0.297	0.054 [-0.050-0.227]	0.219
	PH (n=12)	-0.039 [-0.136-0.204]	>0.99	0.023 [-0.092-0.198]	0.677	-0.018 [-0.135-0.300]	0.806
APAP#1+1h	PPPD (n=7)	0.007 [-0.018-0.161]	0.469	0.065 [0.053-0.287]	0.031	0.220 [0.060-0.349]	0.031
	PH (n=12)	-0.049 [-0.110-0.022]	0.339	0.030 [-0.053-0.108]	0.339	-0.015 [-0.075-0.109]	0.677
After resection	PPPD (n=7)	-0.038 [-0.211-0.107]	0.563	0.126 [0.026-0.451]	0.063	0.185 [-0.002-0.418]	0.219
	PH (n=12)	-0.138 [-0.276-0.103]	0.219	0.100 [-0.219-0.477]	0.469	-0.056 [-0.109-0.380]	0.688

Data are depicted as median with interquartile range

OPH VA gradients		PDV	*p*-value	Liver	*p*-value	Splanchnic	*p*-value
APAP#1	PPPD (n=7)	-0.016 [-0.112-0.020]	0.281	0.100 [0.060-0.280]	0.016	0.122 [0.028-0.294]	0.031
	PH (n=12)	0.004 [-0.044-0.037]	0.954	0.030 [-0.035-0.110]	0.143	0.017 [-0.029-0.138]	0.157
APAP#1+1h	PPPD (n=7)	-0.010 [-0.024-0.018]	0.813	0.080 [0.000-0.260]	0.063	0.120 [0.004-0.266]	0.047
	PH (n=12)	-0.020 [-0.045-0.001]	0.106	0.030 [-0.015-0.080]	0.352	0.008 [-0.017-0.064]	0.482
After resection	PPPD (n=7)	0.001 [-0.064-0.013]	0.844	0.090 [0.035-0.410]	0.031	0.096 [0.025-0.406]	0.063
	PH (n=12)	-0.024	0.031	0.040 [-0.020-0.160]	0.172	0.014 [-0.058-0.148]	0.469

Data are depicted as median with interquartile range

PART III

Prevention and monitoring of postoperative liver dysfunction

Chapter 8

Development of a mouse model for postresectional liver failure

Kim M.C. van Mierlo, Cathy Van Himbeeck, Valérie Lebrun, Peter L.M. Jansen, Cornelius H.C. Dejong, Isabelle A. Leclercq, Steven W.M. Olde Damink, Frank G. Schaap

Submitted 2021

ABSTRACT

Background. Postresectional liver failure (PLF) is a dreaded complication after extended liver resection. Post-operative hyperbilirubinemia suggests that impaired hepatobiliary transport with intrahepatic accumulation of harmful cholephiles plays an etiological role. Bile salts serve dual roles as signalling molecules engaged in liver regeneration after partial hepatectomy (PH) and biological detergents. In this study we tested the hypothesis that excessive accumulation of bile salts in the regenerating liver results in PLF.

Methods. Twelve weeks old male C57BL6/J mice were subjected to 70% PH and post-operatively challenged with a diet supplemented with cholic acid (CA, 0.5 or 1.0%; n=5-6 per group) or a control diet. After 48 hours mice were sacrificed, and liver injury, secretory function, and regenerative indices were assessed.

Results. Mice fed a 1.0% CA diet displayed more pronounced weight loss following PH and had a deranged post-operative glucose course. Liver injury (aminotransferase elevations) and impaired hepatobiliary transport function (hyperbilirubinemia) were apparent in the group fed a 1.0% CA diet, but not in animals fed a 0.5% CA diet. No differences in liver mass recovery were observed among groups. However, the percentage of hepatocytes staining positive for the proliferation marker Ki67 were reduced in mice receiving a 1.0% CA diet relative to animals fed a 0.5% CA diet. PH-induced expression of key factors involved in cell cycle progression (e.g., Foxm1b, Cdc25b) was abrogated in the 1.0% CA diet group.

Conclusion. A postresectional challenge with a 1.0% CA diet induces signs of liver injury and defective liver regeneration. A longer duration of the dietary challenge and/or secondary hits may further improve the model. Once validated, it can be used to evaluate pharmaceutical strategies to prevent or treat PLF.

INTRODUCTION

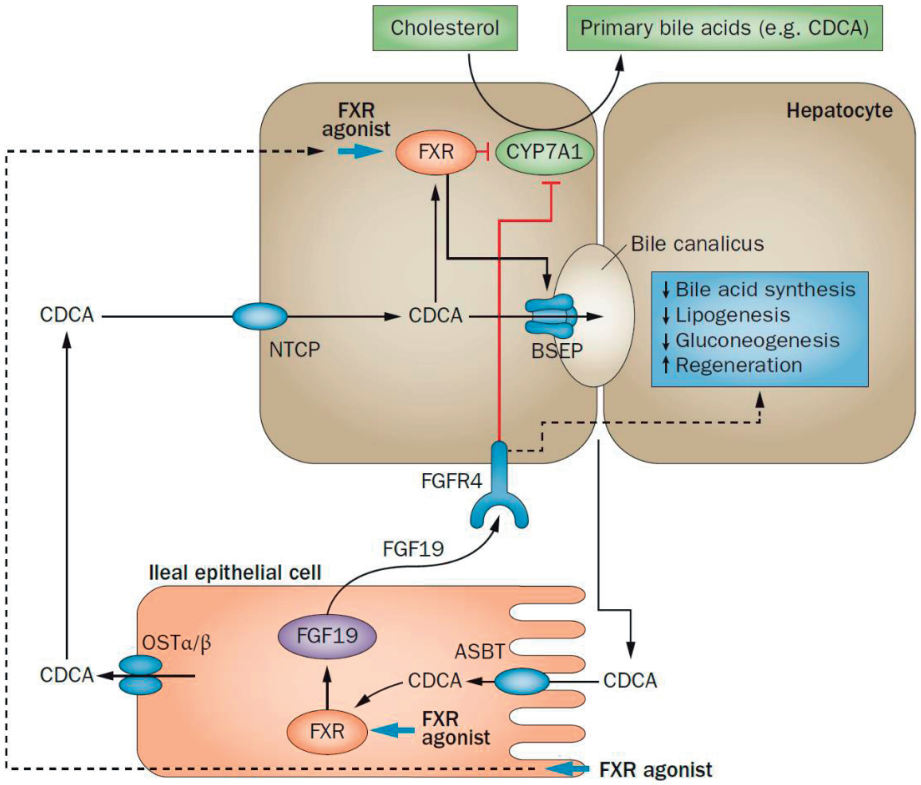
Annually, approximately 7.000 patients develop primary liver cancer or liver metastases in the Netherlands alone.[1,2] Liver resection is the preferred curative treatment for liver malignancies. The feasibility of this procedure is largely dependent on tumour mass extension and localization, resulting in only 15-20% of patients with liver cancer qualifying for resection.[3] Although liver resection is a relatively safe intervention, it is still associated with a mortality rate as high as 2-5% in patients with colorectal liver metastasis and up to 10% in patients with peri-hilar cholangiocarcinoma.[4] Up to 75% of this mortality can be attributed to postresectional liver failure (PLF), where the capacity of the remnant liver to regenerate and simultaneously exert its normal functions is hampered due to an inadequate quantity and quality of the residual liver mass.[3,5]

Post-operative elevation of bilirubin, used in all current clinical definitions of PLF, suggests that impairment of hepatobiliary transporters may underlie development of PLF.[5] Moreover, after liver resection, the remnant liver faces a relative overload of bile salts (BS) because the original BS pool passes through a smaller liver remnant that apparently has insufficient spare capacity to properly handle this increment. This results in increased systemic spill-over and elevation of circulating BS.[6] BS are known to bind to and thus activate several nuclear receptors (NRs), including farnesoid X receptor (FXR or NR1H4) and pregnane X receptor (PXR). FXR is highly expressed in the small intestine and liver (Figure 1), but also in the adrenal glands and kidneys.[7] Upon activation, upregulation of target genes of FXR such as the canalicular bile salt export pump (BSEP) and the sinusoidal BS efflux protein SLC51A/B, maintain hepatic BS homeostasis, thus, limiting BS toxicity and subsequent liver injury. Moreover, FXR is responsible for feedback inhibition of hepatic BS synthesis by MAPK- and SHP-dependent repression of *CYP7A1*, via upregulation of ileal (fibroblast growth factor 19, FGF19) and hepatic (small heterodimer partner, SHP) FXR target genes (Figure 1).[7,8] BS uptake by the enterocyte is the trigger for FXR-mediated transcriptional induction of *FGF19* expression, resulting in the enterokine FGF19 (or Fgf15 in rodents) being secreted into the portal circulation. Subsequent binding of FGF19/Fgf15 to FGFR4 on the surface of hepatocytes results in transcriptional repression of *CYP7A1* and lowering of bile salt synthesis (Figure 1).

Mouse studies have shown that *Fgf15* knockout (KO) mice have increased levels of hepatic *Cyp7a1*, both at mRNA as well as protein level, with a parallel increase in enzyme activity.[9] The role of Fgf15 in liver regeneration was demonstrated by Uriarte et al. (2013), who showed the importance of Fgf15 in maintaining BS homeostasis and preventing liver damage and mortality after hepatectomy. Moreover, this study also showed that Fgf15 mediates spontaneous liver growth, *viz.* in the absence of a surgical trigger, by a diet containing the bile salt cholic acid.[10]

Systemic and intrahepatic accumulation of bile salts are considered a causative factor in acute liver failure (ALF)[3], as observed in mice models of extended hepatectomy (85% liver resection).[11] In these models, the relative overload of BS after hepatectomy caused hepatocellular injury leading to impaired liver regeneration and increased mortality.[11,12] We hypothesized that excessive accumulation of BS in the regenerating liver is the actual culprit in PLF. To test this hypothesis, we aimed to develop a mouse model of PLF by inducing BS overload in the regenerating liver.

Figure 1. Enterohepatic actions of FXR and FGF19



Bile acids (here exemplified as CDCA) are produced in the liver. Bile acids are conjugated and thence referred to as bile salts, and are secreted into the canalicular space for eventual release in the small intestinal lumen. Bile salts are reabsorbed in the terminal ileum. Here, they bind and activate FXR, and this stimulates the production of endocrine acting FGF19. In the liver, FGF19 signalling causes repression of bile acid synthesis. Reclaimed bile salts return to the liver via the portal circulation. In the liver, bile salts activate FXR to regulate (along with FGF19) a multitude of processes, including liver regeneration. BS, bile salt(s); FXR, farnesoid X receptor; MAPK, mitogen activated protein kinase; ASBT, apical sodium-dependent bile salt transporter; BSEP, bile salt export pump; CDCA, chenodeoxycholic acid; CYP7A1, cholesterol 7α-hydroxylase; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; NTCP, sodium-taurocholate co-transporting polypeptide; OST, organic solute transporter; SHP, small heterodimer partner. *Figure copied with permission from the publisher from Schaap, FG. et al. Bile acid receptors as targets for drug development. Nat Rev Gastroenterol Hepatol 2014; 11(1): 55-67*

MATERIALS AND METHODS

Animals

Male C57BL6/J mice (N=18, 11 weeks old) were obtained from Janvier Labs (Le Genest-Saint-Isle, France) and housed in the animal facility of the Université catholique de Louvain (UCL; Brussels, Belgium). The animals were kept under controlled conditions with exposure to a 12-h light/12-h dark cycle and a constant temperature of 20-22°C. Animal experiments and care were conducted in accordance with European regulations and FELASA guidelines for humane care for laboratory animals provided by UCL. Postoperative welfare was assessed with a welfare scoring sheet (Supplemental table 1). The study protocol was approved by the university ethics committee. All mice were fed standard chow (SAFE diets A03 Augry, France) for one week, after which they were subjected to 70% PH (T=0). The procedure was performed essentially according to the protocol of Mitchell and Willenbring (2008), whereby in this study only a small abdominal incision was made, and the gallbladder was removed simultaneously.[13] After the weight of excised median and lateral lobes ('anterior/resected lobes', ~70% of liver mass) was recorded, they were fixed in 4% formalin or snap-frozen in liquid nitrogen and stored at -80°C until analyses. After resection, mice (N=5-6 per group) were fed the same diet supplemented with 0.0%, 0.5% or 1.0% cholic acid (CA; Custom diet, Harlan Laboratories). Mice were sacrificed by exsanguination 48 hours after 70% PH. One mouse (0.0% CA diet) was excluded from the analyses based on elevated liver enzymes, deviating gene expression levels and elevated liver bile salt levels, most likely resulting from a technical failure of the surgical procedure.

Sample preparation

Mice were anesthetized via intraperitoneal injection with ketamine/xylazine at the time of sacrifice. Ten minutes later, the abdominal cavity was opened via midline incision and blood was drawn by portal vein puncture, kept on ice, and serum was prepared and stored at -80°C until further use. After harvesting and weighing the liver ('posterior lobes'), the ileum (distal ⅓ part of the small intestine) was also harvested and both tissues were fixed in 4% formalin, or snap-frozen in liquid nitrogen and stored at -80°C until analysis.

Liver mass recovery

The rate of liver mass recovery was estimated using the following formula:

$$\textit{Liver mass recovery}\ (\%) = 100 \times \frac{M_s}{M_t}$$

where M_s is the liver weight at sacrifice, and M_t is the total liver mass before PH (estimated by dividing the mass of resected segments by 0.7).

Biochemical analyses

Body weight (g) of the animals was monitored daily during weekdays at set time points. Blood glucose levels (mg/dL) were measured in blood drawn from the lateral tail vein (Accu-chek Aviva, Roche diagnostics, Mannheim, Germany) every 4 hours after PH, with a final measurement 2 hours prior to sacrifice. Serum aminotransferases (ALT, AST) and bilirubin were determined 48 hours after PH via automated procedures (Department of Bio-Medical Chemistry and Clinical Biology, St Luc University Hospital, Brussels, Belgium).

Immunohistochemistry

Hepatocyte proliferation was assessed via Ki67 immunohistochemical staining. Firstly, liver tissue was fixed in 4% formalin, dehydrated in graded ethanol, and embedded in paraffin to allow the creation of serial tissue sections of 4 µm thickness. Mouse monoclonal antibody against Ki67 (dilution 1:50; Code No. M7249, Dako, Glostrup, Denmark) was used. Anti-mouse Envision system (Dako) was used for secondary detection. For visualization, the 3,3'-Diaminobenzidine (DAB) Substrate-Chromogen System (Dako) was used. Nuclei were counterstained with haematoxylin. The proliferative index (%) was measured by dividing the amount of Ki67 positive hepatocyte nuclei by the total number of hepatocyte nuclei in four high-power (40x) fields.

Total bile salt assay

A 5% liver homogenate was made by homogenizing ca. 50 mg of tissue in 1 mL of 75% ethanol by means of a Mini-Beadbeater (Biospec Products, Bartlesville, USA). Thereafter, BS were extracted by incubating the homogenates for 2 hours at 50°C.[14] Supernatant was collected after centrifugation (10 min., 20620*g* at 4°C). Total BS in liver extract or serum was quantified with an enzymatic assay according to the manufacturer's protocol (Total bile acid assay, Diazyme Laboratories, Dresden, Germany). The amount of BS present in liver extracts was normalized to wet liver mass.

Real-time polymerase chain reaction

Expression levels of genes involved in BS homeostasis, cell cycle regulation, and proliferation, were measured in liver and ileum samples via RT-qPCR. To study the effect of PH on gene expression per se, non-regenerating liver samples (Resected lobe, T=0h) were included. RNA was isolated from a resected liver lobe (T=0h), the regenerating lobe at sacrifice (T=48h), as well as ileum tissue (T=48h), with TRI reagent solution according to the manufacturer's protocol (Sigma-Aldrich, St. Louis, Missouri). The concentration and purity of RNA were determined by measuring absorbance with the NanoDrop 1000A spectrophotometer (Thermo Scientific). After isolation, RNA was treated with DNAse (Promega, Madison, Wisconsin) to degrade residual genomic DNA, efficiency of DNAse

treatment was verified by PCR. Next, 750 ng total RNA was reverse transcribed to form cDNA according to the manufacturer's protocol (SensiFAST™ cDNA Synthesis Kit, Bioline, Luckenwalde, Germany). Real-time PCR analyses were performed on a MyiQ Single-Color Real-Time PCR detection system (Bio-Rad, Veenendaal, the Netherlands), employing SYBR Green chemistry (SensiMix™ SYBR® & Fluorescein Kit, Bioline). PCR reactions contained 2 µL diluted cDNA sample (corresponding to 7.5 ng total RNA) in a total volume of 10 µL. qPCR data was analysed using LinReg software.[15] Results were normalized using *Rplp0* as a reference gene. Data are expressed as fold expression relative to the median expression in the control group (value set at 1.0). Supplemental table 2 summarizes sequences of primer pairs used for RT-qPCR analyses.

Statistical analysis

The experimental results were statistically analysed using IBM SPSS statistics version 22. Friedman test with Dunn's multiple comparisons testing was used to analyse the evolution of body weight and glycaemia during the study period for all groups (N=5-6 per dose group). Pre- (day -5 until T=0) and post-operative (from T=0 until sacrifice) trajectories were analysed separately. Between-group comparison of glucose levels at each time point, was performed with a Kruskal-Wallis test. The effect of PH (resected lobes at T=0 [N=9] vs. T=48 regenerating lobes of 0.0% CA group [N=5]) was analysed via a Mann-Whitney U test. *P-values* ≤0.05 were considered statistically significant. Differences between the three dose groups (N=5-6 per group) were analysed by means of a Kruskal-Wallis test. If the Kruskal-Wallis test showed significance ($p \leq 0.05$), post-hoc Mann-Whitney U tests were performed between all groups with Bonferroni-Holm correction for multiple comparisons. For visual purposes, biochemical data in graphs is depicted as mean ± SEM. All experimental data on gene expression is graphically presented as median with interquartile range.

RESULTS

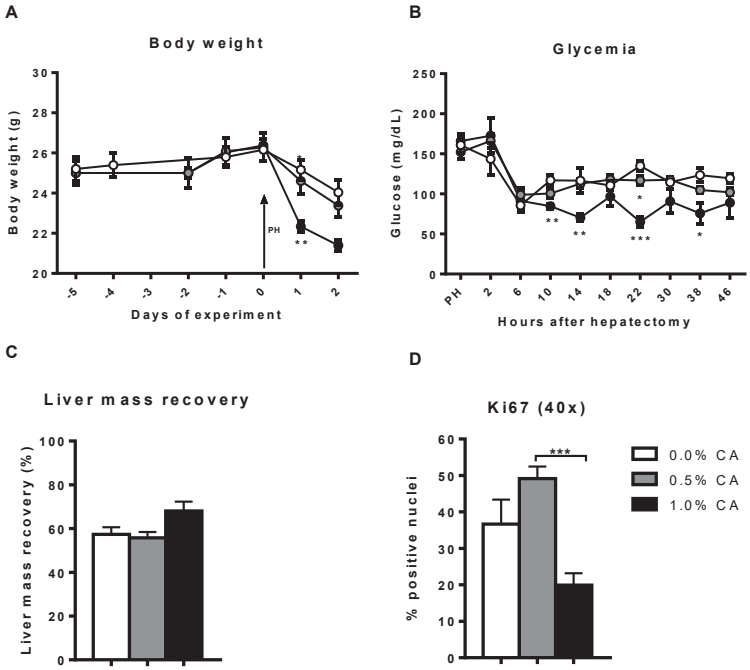
Effect of a CA diet on evolution of body weight, glycaemia, and liver regeneration.

During the pre-operative course in which mice were fed normal chow, significant weight gain was observed in time (p_{time} <0.001-0.0370), Figure 2A). PH resulted in a decrease in body weight in all groups (p_{time}<0.001). At postoperative day one, the highest dose group suffered from more pronounced weight loss compared to the 0.0% and 0.5% CA diet groups (p=0.016 resp. p=0.009). Before PH, glucose levels were not different between the three groups (data not shown). PH induced a transient decrease in glucose levels in all groups (p_{time}=0.005 for 0.0% CA, p_{time}<0.001 for 0.5 and 1.0% CA), with mice fed a 1.0%

CA diet showing an overall deviating course compared to the two other groups. Serum glucose was significantly reduced in mice receiving diet containing 1.0% CA, at T=10, T=14, T=22 and T=38h (Figure 2B).

No differences in liver mass recovery (based on wet liver mass) were observed between the three groups (Figure 2C). However, mice fed a 1.0% CA diet were found to have a lower percentage of proliferating hepatocytes in comparison with mice fed a 0.5% CA diet (49% vs. 20%, p=0.002, Figure 2D).

Figure 2. Metabolic derangement and abrogated hepatocyte proliferation in hepatectomized mice fed a diet containing 1.0% cholic acid



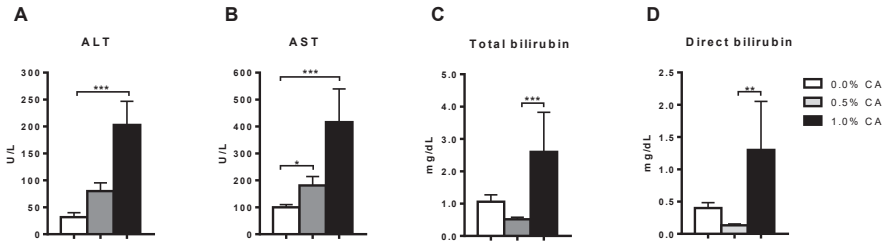
Mice (n=5-6 per group) were subjected to 70% partial hepatectomy (PH) and post-operatively challenged with a diet containing 0.0%, 0.5% or 1.0% cholic acid. Mice were sacrificed at 48 hrs after PH. Experimental courses of body weight **(A)** and blood glucose **(B)**. Data are presented as mean with SEM. Regeneration after PH was assessed by recovery of liver mass **(C)** and immunohistochemical analysis of hepatocyte proliferation **(D)**. Data are presented as mean with SEM. ***p<0.005; **p<0.01; *p<0.05; PH, partial hepatectomy; CA, cholic acid

Effect of a CA diet on markers for liver injury and secretory function

Mice fed a 1.0% CA diet had significantly increased levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in comparison to the 0.0% CA diet group (both p=0.004, Figure 3A, B). Moreover, serum AST was increased in the mice fed a 0.5% diet compared to the 0.0% CA diet group. Regarding total and direct bilirubin

levels, mice fed a 1.0% CA diet showed higher levels than mice fed a 0.5% CA diet (p=0.002 resp. p=0.007; Figure 3C, D), indicating an impaired secretory function of the liver in the highest dose group. Moreover, mice in the 1.0% CA group did clinically worse than the other groups as observed by reduced physical activity, hunched posture, squinted eyes and piloerection. At sacrifice, their livers appeared yellow, and ascites was observed (Supplemental figure 1).

Figure 3. Elevated liver enzymes and hyperbilirubinemia in hepatectomized mice fed diet containing 1.0% cholic acid



Mice were subjected to 70% partial hepatectomy (PH) and post-operatively challenged with diet containing 0.0%, 0.5% or 1.0% cholic acid (n=5-6 per group). Mice were sacrificed at 48 hrs after PH. Liver injury after hepatectomy was assessed by serum liver enzymes **(A, B)** and secretory function **(C, D)**. Data are presented as mean with SEM. ***p<0.005; **p<0.01; *p<0.05; CA, cholic acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase

Influence of a CA diet on hepatic and serum bile salt content

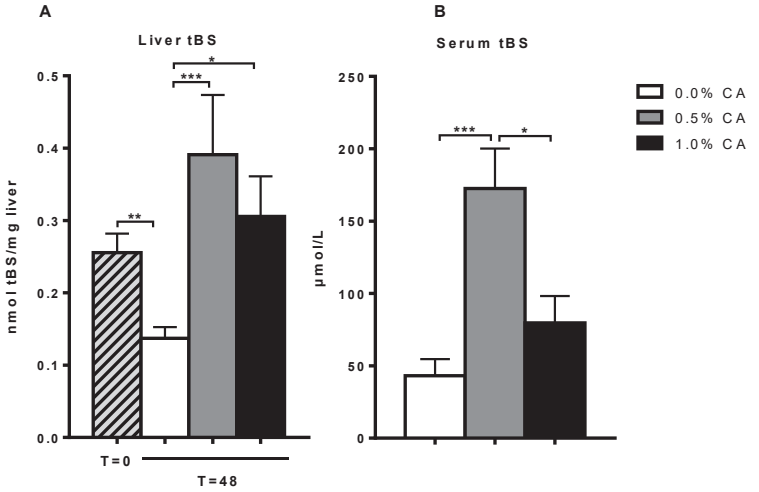
After PH, hepatic BS content dropped significantly (T=0 vs. T=48 hrs in the 0.0% CA group, p=0.009, Figure 4). After PH, mice fed a 0.5% and 1.0% CA diet both showed significantly higher hepatic BS levels compared to a 0.0% CA diet (p=0.004 resp. p=0.017; Figure 4A). Serum BS levels were increased in the mice fed a 0.5% CA diet in comparison to the control group and mice fed a 1.0% CA diet. (p=0.004 resp. p=0.015, Figure 4B).

Bile salt synthesis and regulation

Maintenance of bile salt homeostasis in the remnant liver is a prerequisite for normal progression of liver regeneration after PH. Notably, hepatic BS accumulation was accompanied by reduced hepatocyte proliferation in mice receiving diet with 1.0% CA. Expression of genes engaged in different aspects of bile salt homeostasis was determined to investigate this further.

Expression levels of genes involved in BS synthesis and regulation thereof, were measured in liver and ileum. The regulatory genes include ileal *Fgf15* and hepatic *Fxr* and its direct target gene *Shp*, while genes engaged in BS synthesis include *Cyp7a1*, *Cyp8b1* and *Cyp7b1* (liver). PH induced a downregulation in hepatic gene expression of *Fxr* and *Shp* (p<0.001

Figure 4. Metabolic derangement and abrogated hepatocyte proliferation in hepatectomized mice fed a diet containing 1.0% cholic acid



Mice were subjected to 70% partial hepatectomy (PH) and post-operatively challenged with diet containing 0.0%, 0.5% or 1.0% cholic acid (n=5-6 per group). Mice were sacrificed at 48 hrs after PH. Total bile salt levels were measured in liver before and after hepatectomy **(A)** and in serum after hepatectomy **(B)**. Data are presented as mean with SEM. ***p<0.005; **p<0.01; *p<0.05; CA, cholic acid; tBS, total bile salt(s)

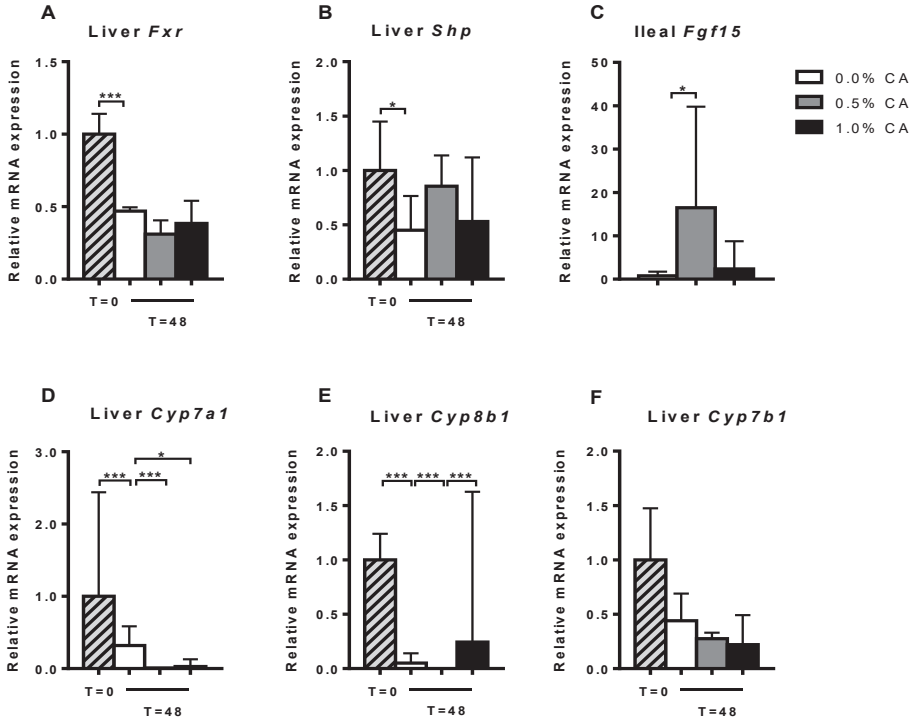
resp. p=0.029) in the 0.0% CA diet group (Figure 5A, B). No differences in expression of *Fxr* and *Shp* in the different diet groups were observed after PH. After PH, gene expression of ileal *Fgf15* was elevated 16.5-fold in mice fed a 0.5% CA diet in comparison with a 0.0% CA diet (p=0.011) (Figure 5C). However, no significant difference was found in comparison with the 1.0% CA group (p=0.628).

In concordance with previous studies, PH induced a 4.1-fold reduction in *Cyp7a1* expression levels in mice fed a 0.0% CA diet (p<0.001; Figure 5D), and further downregulation occurred in the 0.5% and 1.0% CA diet groups (p=0.004 resp. p=0.015). *Cyp8b1* expression levels followed the same pattern, although expression after PH was higher in mice fed 1.0% CA compared to those fed 0.5% CA (p=0.002) (Figure 5E). *Cyp7b1* gene expression was neither influenced by PH nor by CA feeding (Figure 5F).

Bile salt uptake and export

Expression levels of genes engaged in the uptake and export of BS in liver and ileum were studied next (Figure 6). Regarding uptake transporters, a difference was only seen for hepatic *Ntcp* expression, namely a 3-fold reduction after PH (p<0.001), with no additional effects of CA feeding (Figure 6A). Regarding Fxr-regulated transporters engaged in basolateral (Slc51b) or canalicular (Bsep) BS efflux, PH caused marked upregulation (4-fold) of hepatic *Slc51b* expression (p<0.001) (Figure 6B). This was further induced in

Figure 5. Suppression of bile salt synthetic genes in hepatectomized mice

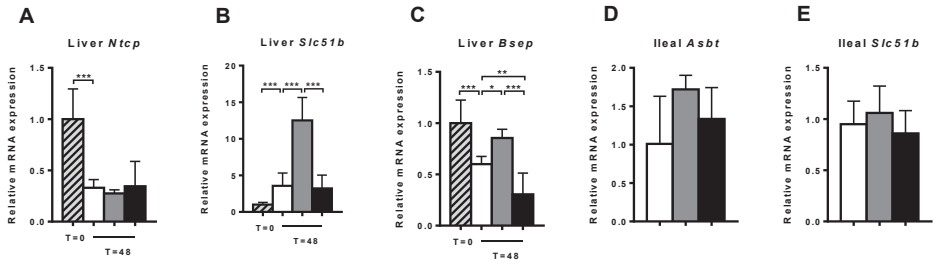


Mice were subjected to 70% partial hepatectomy (PH) and post-operatively challenged with diet containing 0.0%, 0.5% or 1.0% cholic acid (n=5-6 per group). Mice were sacrificed at 48 hrs after PH. Bile salt homeostasis was assessed after PH by activation of the Fxr-pathway in liver **(A, B)** and ileum **(C)**. De novo bile salt synthesis was assessed by cytochrome P450 liver enzymes from the classical **(D, E)** and acidic pathway **(F)**. Values are expressed relative to the median expression in the control group. ***p<0.005; *p<0.05; CA, cholic acid; Fxr, farnesoid X receptor; Shp, small heterodimer partner; Fgf, fibroblast growth factor; Cyp7a1, cholesterol 7α-hydroxylase; Cyp8b1, sterol 12-α-hydroxylase; Cyp7b1, oxysterol 7α-hydroxylase

mice fed a 0.5% CA diet, which had 3.1 resp. 3.9-fold higher *Slc51b* expression in relation to mice fed a 0.0% CA diet or a 1.0% CA diet (p=0.004 resp. p=0.002). PH induced a slight reduction (1.7-fold) in expression of *Bsep* (p=0.004) and feeding the mice 0.5% CA diet partially restored expression (p=0.017 vs. 0.0% CA diet) (Figure 6C). In contrast, the 1.0% CA diet repressed *Bsep* expression relative to mice receiving a 0.0% or 0.5% CA diet (p=0.008 resp. p=0.002).

No significant differences were seen in hepatic expression of transporters mediating canalicular secretion of bilirubin (*Mrp2*) and phospholipids (*Mdr3*), and basolateral secretion of BS (*Mrp3*, *Mrp4*) after PH (Supplemental figure 2). In addition, CA feeding had no effect on expression of genes involved in intestinal uptake (*Asbt*) and secretion (*Slc51b)* of BS (Figure 6D, E).

Figure 6. Expression of genes involved in hepatic and intestinal bile salt transport



Mice were subjected to 70% partial hepatectomy (PH) and post-operatively challenged with diet containing 0.0%, 0.5% or 1.0% cholic acid (n=5-6 per group). Mice were sacrificed at 48 hrs after PH. Hepatocellular bile salt transport was assessed by gene expression of proteins engaged in sodium-dependent **(A)** and -independent **(B)** hepatocellular bile salt uptake, and canalicular bile salt export **(C)**. Ileal bile salt uptake was assessed by gene expression of apical **(D)** and basolateral **(E)** transporters. Values are expressed relative to the median expression in the control group. ***p<0.005; **p<0.01; *p<0.05; CA, cholic acid; Ntcp, sodium-taurocholate cotransporting polypeptide; Slc51b, organic solute transporter beta; Bsep, bile salt export pump; Asbt, apical sodium-dependent bile transporter

Cell cycle regulation and proliferation

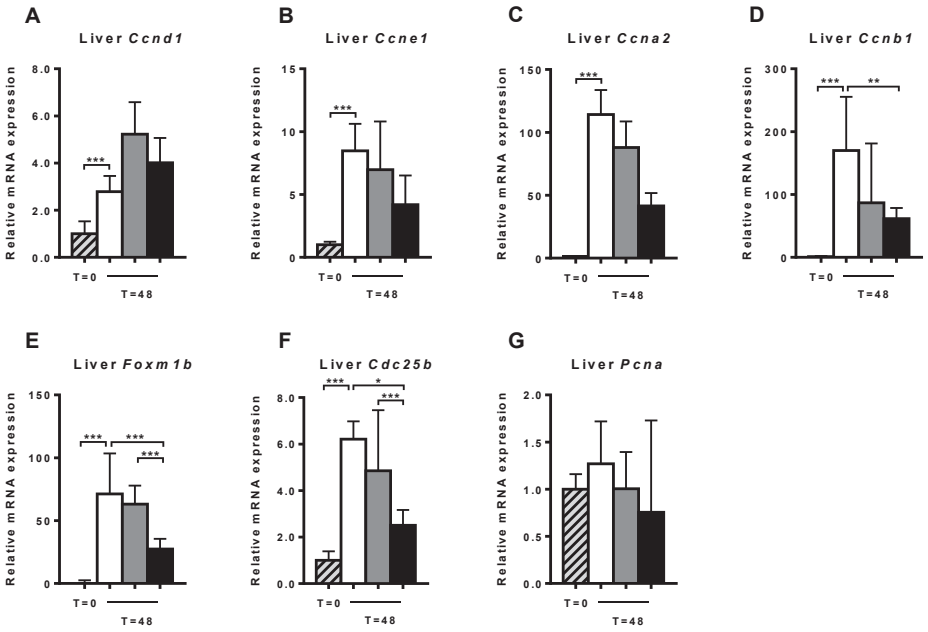
Since hepatocyte proliferation was impaired in hepatectomized mice receiving a 1.0% CA diet, we examined hepatic expression of genes engaged in cell cycle regulation and proliferation. PH resulted in marked upregulation of cell cyclins *Ccnd1* (2.6-fold), *Ccne1* (8.8-fold), *Ccna2* (106-fold) and *Ccnb1* (159-fold) in control mice (p<0.002; Figure 7A-D), and with exception for *Ccnb1,* the extent of induction was comparable between diet groups. Mice fed a 1.0% CA diet had lower *Ccnb1* expression levels than mice fed a 0.0% CA diet (p=0.009).

Regarding expression of the cell cycle regulating transcription factor *Foxm1b*, whilst expression was virtually undetectable in quiescent liver, PH caused a marked induction (64-fold) (p<0.001; Figure 7E). When comparing the different dose groups, the 1.0% CA group showed reduced PH-induced *Foxm1b* expression compared to both the 0.0% and 0.5% CA diet group (both p=0.004). Gene expression level of cell division cycle 25b (*Cdc25b*), a direct target gene of *Foxm1b* and required for entry into mitosis, was induced by PH (p<0.001) (Figure 7F). Analogous to *Foxm1b*, the 1.0% CA group had an abrogated induction of *Cdc25b* compared to mice receiving a 0.0% or 0.5% CA diet (p=0.017 resp. p=0.002). PH or CA feeding had no effect on expression of proliferating cell nuclear antigen (*Pcna*) (Figure 7G).

DISCUSSION

PLF is a serious complication following liver resection with high morbidity and mortality. To find an appropriate therapy to treat and/or prevent the occurrence of liver failure

Figure 7. Abrogated cell cycle progression in hepatectomized mice fed a diet containing 1.0% cholic acid



Mice were subjected to 70% partial hepatectomy (PH) and post-operatively challenged with diet containing 0.0%, 0.5% or 1.0% cholic acid (n=5-6 per group). Mice were sacrificed at 48 hrs after PH. Cell cycle progression was assessed by gene expression of enzymes involved in cell cycle regulation **(A-D)**, entry into mitosis **(E, F)** and DNA synthesis **(G)**. Values are expressed relative to the median expression in the control group. ***p<0.005; **p<0.01; *p<0.05; CA, cholic acid; Ccn, cyclins; Fox, forkhead box; Cdc, cell division cycle; Pcna, proliferating cell nuclear antigen

after resection, an animal model of PLF is of great value. We hypothesized that excessive accumulation of BS in the regenerating liver remnant is the actual culprit in PLF. To test this, we induced BS overload in the regenerating liver of mice by feeding them a CA diet after 70% PH. Concentrations of CA in the diet ranged from 0.0 to 1.0%, and mice were sacrificed around the time of maximal hepatocyte proliferation (normally peaking between 36-48 hrs).

Some interesting findings were observed. Mice in the highest dose group had poorer 'clinical' performance (i.e., squinted eyes, reduced physical activity) as indicated by our welfare assessment (Supplemental figure 1), with overall decreased glucose levels after PH, and more pronounced body weight loss in the postresectional course (Figure 2A, B). Moreover, in this group, assessment of injury (aminotransferases) and secretory function (total bilirubin) of the liver revealed hepatic injury and an impaired secretory function (Figure 3). Although no effect of a 1.0 % CA diet was seen on liver mass recovery, impaired hepatocyte proliferation was noted (Figure 2C, D).

In contrast, despite a cholic acid-supplemented diet, mice fed a 0.5% CA diet seemed to perform similar or even better than the 0.0% CA diet group in terms of hepatocyte proliferation. Ileal *Fgf15* was upregulated in mice fed a 0.5% CA diet compared to a 0.0% CA diet (Figure 5C). No signs of liver injury and a maintained hepatocytic proliferative capacity were seen in the 0.5% CA group. Since FGF19/Fgf15 has been identified as a direct hepatic mitogen, maintained proliferative capacity in the 0.5% CA group may relate to enhanced ileal *Fgf15* expression.[10]

In our study, a reduction in hepatic BS content was seen in control mice 48 hrs after PH (Figure 4A). We studied gene expression of Fxr-regulated genes to obtain a mechanistic explanation for this observation. PH is known to result in a relative overload of endogenous BS in the remnant liver directly after PH, likely resulting in a compensating rapid increase in bile salt signalling with activation of Fxr and induction of Fxr target genes, such as *Shp* and *Slc51b*.[16] Previously, Uriarte et al. demonstrated a transient increase in intrahepatic BS levels 24 hours after PH, followed by a decrease to values almost equal to baseline levels after 48 hours.[10] This is in accordance with Huang et al., who showed that PH induced a decrease in hepatic BS content in mice after 48 hours.[17] The decreased hepatic BS content 48 hours after PH may be linked to Fxr/Fgf15-mediated repression of bile salt synthesis (*Cyp7a1*) and downregulation of the hepatocytic BS uptake transporter *Ntcp* in reaction to the relative overload,[7] which was also observed in our study (Figure 5D, 6A).

Although an elevation in hepatic BS content was seen in both CA-fed groups compared to a 0.0% CA diet, no difference was found between the various dose groups. These results were not in accordance with the expectation that a 1.0% CA diet would result in more accumulation of BS in the remnant liver and consequent impaired liver mass regrowth (regeneration). Possible differences in hepatic bile salt composition, an important determinant of cytotoxicity, have not been assessed. Likewise, hepatic BS measurements are inevitably based on homogenates, and information on the actual spatial localization (i.e., within the hepatocytes, within the biliary network) of BS is not available. Yet, it is plausible that accumulation of BS within the hepatocytes, negatively impacts hepatocyte proliferation. Divergent changes in *Bsep* expression in the CA diet groups, favouring localization of BS within the biliary network in the group (0.5% CA diet) that has highest percentage of proliferating hepatocytes, supports this idea. To determine the spatial distribution of a variety of bile salts in liver sections and therewith investigate its local functions, innovative techniques such as matrix-assisted laser desorption/ionization-mass spectrometry imaging (MALDI-MSI) have proven profitable.[18]

CA feeding led to an elevation of serum BS in the 0.5% CA group only. This can be interpreted as adaptive mechanisms that serve to maintain low intracellular BS levels, e.g.,

enhanced basolateral (via Slc51b) and canalicular export (via Bsep), being functional in the 0.5% CA diet group. By extension, these protective mechanisms may have failed in the 1.0% CA diet group. Again, data on spatial localization of BS in the CA groups, would be informative.

Postresectional hepatocellular proliferation proceeds through tightly regulated transitions which are, amongst others, controlled by cell cycle regulatory genes. PH induced an upregulation in gene expression of all cyclins that were studied, as well as Foxm1b and Cdc25b. This is in accordance with the literature, and the consequence of cell cycle re-entry upon hepatic resection.[19] Importantly, mice fed a 1.0% CA diet had lower PH-induced Ccnb1, Foxm1b and Cdc25b levels compared to mice fed a 0.0% or 0.5% CA diet (Figure 7), and this translates to the reduced percentage of proliferating (i.e., Ki67+) hepatocytes (Figure 2D). This means that cell cycle progression is hampered in the 1.0% CA group. Impaired cell proliferation could be due to initiation of liver injury that interferes with proper regeneration and/or outbalances cell gain. In contrast with the study of Uriarte et al., we did not find any differences in Pcna at the mRNA level (Figure 7G).

After PH, we regard liver mass recovery as one of the most important regeneration indices. Although calculation of liver mass recovery had been used in previous studies as index for liver regeneration,[20,21] it is a rough estimate based on wet weight that assumes removal of exactly 70% of the liver in all cases and does not consider unrelated causes of liver mass gain. Especially regarding the poor clinical performance of mice fed a 1.0% CA diet, for instance hepatic oedema may have affected wet liver weight measurement. In this regard, direct indices to assess hyperplasia of the liver, such as Ki67 staining that was employed here, are more informative.

In conclusion, a postresectional BS challenge of 1.0% CA induced signs of liver injury and resulted in impaired liver regeneration. Based on these data, we can conclude that the approach (postresectional CA-challenge) to establish a PLF model looks promising, but modifications to the protocol are required. To test if the pre-damaged liver is extra susceptible for (bile salt) injury, we could apply the current model in mice with cholestatic liver disease due to occlusion of the common bile duct.

REFERENCES

1. IKNL – Cijfers over kanker. Incidentie | Lever en intrahepatische galwegen L, 2018.
2. IKNL – Colorectale levermetastasen Lr, Versie: 1.0 (2006).
3. van den Broek MA, Olde Damink SW, Dejong CH, et al. Liver failure after partial hepatic resection: definition, pathophysiology, risk factors and treatment. *Liver int* 2008; **28**:767-780.
4. Franken LC, Schreuder AM, Roos E, et al. Morbidity and mortality after major liver resection in patients with perihilar cholangiocarcinoma: A systematic review and meta-analysis. *Surgery* 2019; **165**:918-928.
5. van Mierlo KM, Schaap FG, Dejong CH, Olde Damink SW. Liver resection for cancer: New developments in prediction, prevention and management of postresectional liver failure. *J Hepatol* 2016; **65**:1217-1231.
6. Doignon I, Julien B, Serriere-Lanneau V, et al. Immediate neuroendocrine signaling after partial hepatectomy through acute portal hyperpressure and cholestasis. *J Hepatol* 2011; **54**:481-488.
7. Jonker JW, Liddle C, Downes M. FXR and PXR: potential therapeutic targets in cholestasis. *J Steroid Biochem Mol Biol* 2012; **130**:147-158.
8. Schaap FG, Trauner M, Jansen PL. Bile acid receptors as targets for drug development. *Nat Rev Gastroenterol Hepatol* 2014; **11**:55-67.
9. Inagaki T, Choi M, Moschetta A, et al. Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. *Cell Metab* 2005; **2**:217-225.
10. Uriarte I, Fernandez-Barrena MG, Monte MJ, et al. Identification of fibroblast growth factor 15 as a novel mediator of liver regeneration and its application in the prevention of post-resection liver failure in mice. *Gut* 2013; **62**:899-910.
11. Zhang L, Huang X, Meng Z, et al. Significance and mechanism of CYP7a1 gene regulation during the acute phase of liver regeneration. *Mol Endocrinol* 2009; **23**:137-145.
12. Perez MJ, Briz O. Bile-acid-induced cell injury and protection. *World J Gastroenterol* 2009; **15**:1677-1689.
13. Mitchell C, Willenbring H. A reproducible and well-tolerated method for 2/3 partial hepatectomy in mice. *Nat Protoc* 2008; **3**:1167-1170.
14. Modica S, Murzilli S, Moschetta A. Characterizing Bile Acid and Lipid Metabolism in the Liver and Gastrointestinal Tract of Mice. *Curr Protoc Mouse Biol* 2011; **1**:289-321.
15. Ramakers C, Ruijter JM, Deprez RH, Moorman AF. Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. *Neurosci Lett* 2003; **339**:62-66.
16. Fan M, Wang X, Xu G, Yan Q, Huang W. Bile acid signaling and liver regeneration. *Biochim Biophys Acta* 2015; **1849**:196-200.
17. Huang W, Ma K, Zhang J, et al. Nuclear receptor-dependent bile acid signaling is required for normal liver regeneration. *Science* 2006; **312**:233-6.
18. Flinders B, Huizing LRS, van Heerden M, et al. Cross-Species Molecular Imaging of Bile Salts and Lipids in Liver: Identification of Molecular Structural Markers in Health and Disease. *Anal Chem* 2018; **90**:11835-11846.
19. Chauhan A, Lorenzen S, Herzel H, Bernard S. Regulation of mammalian cell cycle progression in the regenerating liver. *J Theor Biol* 2011; **283**:103-112.
20. Selzner M, Clavien PA. Failure of regeneration of the steatotic rat liver: disruption at two different levels in the regeneration pathway. *Hepatology* 2000; **31**:35-42.
21. Leclercq IA, Vansteenberghe M, Lebrun VB, et al. Defective hepatic regeneration after partial hepatectomy in leptin-deficient mice is not rescued by exogenous leptin. *Lab Invest* 2006; **86**:1161-1171.

Supplemental data

Table 1. Postoperative welfare sheet

Postoperative day	___	___	___	___	___	___	___	__
Time								
Weight								
Temperature								
Analgesic								
Score per point. Normal = 0, deterioration can go up to 1,2 or 3 points								
Activity								
Behavior								
Gait								
Posture								
Physical condition								
Fur/skin								
Eyes								
Hydration								
Breathing								
Faeces/urine								
Surgical wound								
Oedema/ascites								
Necrosis								

Welfare scores

Description	0	1	2	3
Activity	Normal	Isolated, less active	Inactive	Somnulent, stupor, coma, lifeless
Behavior (Arching of back)	Normal	Back arching, twitching, shivering Once/10 min	Back arching, twitching, shivering	Stereotype behavior, auto mutilation, aggressive behavior
Gait	Normal	Mildly uncoordinated/ Abnormality	Uncordinated walking on toes, limping	Paralysis, limp, convulsions, tremor.
Posture	Normal	Huddled up, stretching	Imbalance, twitching	Fall over, circle
Physical condition	Normal	BC2= condition	BC5= obese	BC1= emaciated BC6=extreme obese
Fur/skin	Normal	Dry, rough, not shiny anymore	Piloerection, small wounds, dry white skin	Red/black skin, inflammation, wounds, loss of fur
Eyes	Normal	Not fully open	Less open, dirty	Closed, dirty
Hydration	Normal	Loss of skin elasticity	Reduced skin turgor	Severely reduced turgor + sunken eyes
Breathing	Normal	Fast and superficial	Fast abdominal breathing + audible breathing	Respiratory problems, cyanosis, breathing with open mouth
Faeces/ urine	Normal	Moist faeces, polyurie	Diarrhea, abnormal urine	Uncontrolled diarrhea, bloody stool, obstipation, hematuria
Surgical wound	Normal healing	Sutures intact, slighty red/bloody	Dehiscence of wound, sutures open, fluid secretion	Severe bleeding, wound open, severe redness, necrosis
Oedema	Normal	Mild abnormal fluid collections, swollen appearance	Abnormal large abnormal fluid collections, ascites	Severe large abnormal fluid collections
Necrosis	Normal	Dark skin colouring	Small dark/black spots, burning wounds, blisters	Big black spots, crusts

Sacrifice sheet

	Normal	Abnormal
Abdomen		e.g. bloated
Wound/stitches		e.g. herniation, necrosis, wound dehiscence
Intra-abdominal cavity		e.g. yellow skin, ascites
Liver		e.g. pale, with spots
Stomach/intestines		e.g. ileus, adhesions
Kidney/spleen		e.g. other color (black, pale), increased/decreased size

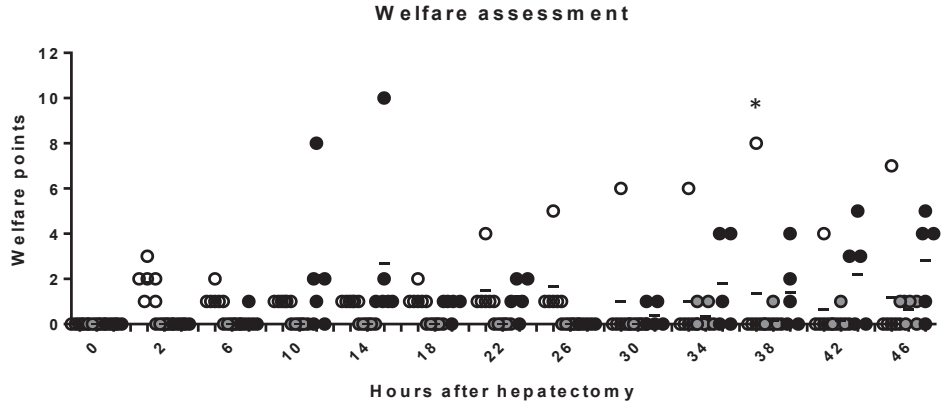
Humane endpoints are defined as: significant weight loss >20%, fever (temperature will only be measured on indication), tachypnoe, significant differences in behavior: lethargia, twitching: random spasms of the muscles can be seen when animals are asleep or inactive in huddled up position, walking: unable to stand on four legs, wobbly walk >6 hours after surgery, huddled up posture: showing a concave abdominal side, signs of severe dehydration, severe diarrhea, severely inflamed surgical wound, cachexia, severe hypoglycaemia <20 >6 hours postoperative

Table 2. Primer sequences real-time PCR

Gene	Primer sequences	
	Forward	*Reverse*
36b4 (Rpl0)	TCGTTGGAGTGACATCGTCTT	TCTGCTCCCACAATGAAGCA
Fxr (Nr1h4)	AAGCTTCCAGGGTTTCAGACA	CTGTGAGCAGAGCGTACTCC
Shp	GGAAGCCAGCAGCGGTACCC	TGCGATGTGGCAGGAGGCAC
Cyp7a1	ACAACCTGCCAGTACTAGATAGC	AGGTGGTCTTTGCTTTCCCA
Cyp8b1	GGTACGCTTCCTCTATCGCC	GAGGGATGGCGTCTTATGGG
Cyp7b1	TCTCTTTGCCGCCACCTTAC	ATACTTCCCCACAAGGAAGACAG
Fgf15	ACTGCGAGGAGGACCAAAAC	CCGAGTAGCGAATCAGCCC
Bsep (Abcb11)	CTATAGCTGCCGCAAAGCAG	AGCTGCACTGTCTTTTCACT
Mrp2 (Abcc2)	ACAACCTGAGCATAGGGCAGA	GCCGCTGTCTAGGACCATTA
Mdr3 (Abcb4)	TCTATGACCCCATGGCTGGA	GTGTTATATTTTTGGGGCAGCGT
Mrp3 (Abcc3)	CTAAGACCAAGACTGAGGCCC	CCAGGATTCGGAACAGGCAA
Mrp4 (Abcc4)	AATGTGGACCCAAGAACGGA	GCAGCAAGACATACGGCTCA
Ntcp (Slc10a1)	AATCCAAGCTGCAGACGCA	TGCAGCAGCCTTGTAGGTAA
Asbt (Slc10a2)	ACAAATGGCCACAAAAAGCGA	ACTGTTCGGCACCTGTACCA
Ostβ (Slc51b)	CCCAGGAACTGCTGGAAGAAA	GGCTGCTTCTTTCGATTTCTGTT
Foxm1b	AGCTAAGGGTGTGCCTGTTC	GGGCTCCTCAACCTTAACCC
Cdc25b	ATTCTCGTCTGAGCGTGGAC	GGCTCACAAAAGTTCGGATGC
Pcna	GGCTTCGACACATACCGCT	AGCTGTACTCCTGTTCTGGGA
Ccna2	AACAGAGTGTGAAGATGCCCT	ATTTAACCTCCATTTCCCTAAGGT
Ccnb1	TAAGGCCGTGACAAAGGCAT	TCGACAACTTCCGTTAGCCT
Ccnd1	CTGCCGAGAAGTTGTGCATC	AAATGAACTTCACATCTGTGGCA
Ccne1	AAGGGAGAGAGACTCGACGG	GGGATGAAAGAGCAGGGGTC

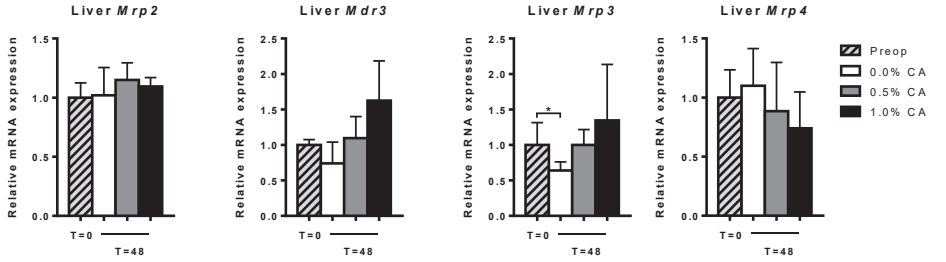
PCR, polymerase chain reaction; Rplp0, acidic ribosomal phosphoprotein P0; Fxr, farnesoid X receptor; Shp, small heterodimer partner; Cyp7a1, cholesterol 7α-hydroxylase; Cyp8b1, sterol 12-α-hydroxylase; Cyp7b1, oxysterol 7α-hydroxylase; Fgf, fibroblast growth factor; Bsep, bile salt export pump; Mrp, multidrug resistance-related protein; Mdr, multidrug resistance protein; Ntcp, sodium-taurocholate co-transporting polypeptide; Asbt, apical sodium-dependent bile transporter; Ost, organic solute transporter; Fox, forkhead box; Cdc, cell division cycle; Pcna, proliferating cell nuclear antigen; Ccn, cyclins

Figure 1. Increased welfare scores in mice fed a 1.0% cholic acid diet



Mice were subjected to 70% partial hepatectomy (PH) and post-operatively challenged with diet containing 0.0%, 0.5% or 1.0% cholic acid (n=5-6 per group). Mice were sacrificed at 48 hrs after PH. Welfare was assessed by a specific score list. Data are presented as median with interquartile range. A higher total of welfare points indicates a lower welfare. *Mouse excluded from analysis due to technical failure of PH; CA, cholic acid

Figure 2. Unaltered gene expression of proteins engaged in basolateral and canalicular secretion of cholephiles in mice fed a diet containing 1.0% cholic acid



Mice were subjected to 70% partial hepatectomy (PH) and post-operatively challenged with diet containing 0.0%, 0.5% or 1.0% cholic acid (n=5-6 per group). Mice were sacrificed at 48 hrs after liver resection. Canalicular secretion of bilirubin **(A)** and phospholipids **(B)**, and basolateral secretion of BS **(C, D)** was assessed by gene expression of its transporters. Data are presented as median with interquartile range. *p<0.05; CA, cholic acid; Mrp, multidrug resistance-related protein; Mdr, multidrug resistance protein

Chapter 9

Obeticholic acid does not stimulate liver regeneration in hepatectomized mice

Kim M.C. van Mierlo, Carola Dahrenmoller, Valérie Lebrun, Peter L.M. Jansen, Cornelius H.C. Dejong, Isabelle A. Leclercq, Steven W.M. Olde Damink, Frank G. Schaap

Submitted 2021

ABSTRACT

Background. Postresectional liver failure (PLF) is a dreaded complication after partial hepatectomy (PH). Data from animal experiments indicate that endogenous ligands (i.e., bile salts) can stimulate liver regeneration and prevent liver injury after PH, via the hepatic Fxr and ileal Fxr-Fgf15 axis. Our aim was to investigate whether exogenous activation of the Fxr pathway with the semi-synthetic bile acid obeticholic acid (OCA) could stimulate postresectional liver regeneration in mice.

Methods. Twelve weeks old male C57BL6/J mice were pre-treated with OCA or vehicle, and after 7 days subjected to 70% PH. Mice were sacrificed at 24, 48 and 72 hrs after PH, and liver injury, secretory function, and regenerative indices were assessed. In the second study, OCA pre-treated mice received oral sucrose supplementation in the postoperative trajectory, and a group of mice receiving intraperitoneal injections of FGF19 was included as a positive control group. Here, mice were sacrificed at 48 hours after PH.

Results. No effect could be detected on liver mass recovery after PH, although responses of Cyp7a1, Cyp8b1 and other Fxr target genes implied general effectiveness of OCA treatment. OCA had no effect on the number of Ki67+ hepatocytes and mitotic figures at 48 hrs after PH. Hepatic bile salt content did not improve around the peak of proliferation at 48 hrs after PH. Besides, welfare of the animals was decreased in OCA-treated animals after PH. After pre-treatment of mice with FGF19, a reduced expression of ileal bile salt-regulated genes Fgf15 and Slc51b indicating FGF19-mediated repression of bile salt synthesis was seen, but this did not stimulate postresectional liver regeneration in mice.

Conclusion. Despite the activation of hepatic and ileal Fxr as shown by induction of its target genes, treatment with OCA or FGF19 did not result in accelerated liver regeneration after PH and liver bile salt content was not influenced. We speculate that bile salt homeostasis and endogenous bile salt signalling is already optimal in unaffected livers for proper progression of regeneration/repair after PH. It would be interesting to study the effect of Fxr agonism on liver regeneration after PH and prevention of PLF in the context of compromised bile salt homeostasis/signalling prior to PH.

INTRODUCTION

Partial hepatectomy is often the preferred curative treatment for hepatobiliary malignancies. However, only 15-20% of patients are eligible for liver resection, mainly due to extensive disease and a predicted insufficient liver remnant.[1] In healthy patients, up to 75% of hepatic volume can be resected.[2] In patients with hepatic functional impairment (e.g., due to steatohepatitis or cirrhosis), a maximum of 60% can be removed. An imbalance between liver volume and quality, with lack of functional recovery after (extended) resection, may lead to postresectional liver failure (PLF). PLF is clinically characterized by hyperbilirubinemia, coagulopathy and hepatic encephalopathy, and occurs in up to 9% of patients, with high lethality.[1] Current clinical practice focuses on preoperative enlargement of future remnant liver volume/function (portal vein embolization[3]), or acceleration of functional hypertrophy of the future remnant liver with novel surgical techniques such as the ALPPS (Associating Liver Partition and Portal vein ligation for Staged hepatectomy) procedure[4,5] to overcome this problem. However, both procedures are not always successful, and complication rates are considerable in case of ALPPS.

Postresectional liver regeneration comprises a complex biological response involving interaction between parenchymal and non-parenchymal cells by means of endo-, angio- and paracrine signalling by cytokines, growth factors and metabolic factors.[6-8] In the past decade, bile salts have transpired as essential signalling molecules in liver regeneration, opening new areas of therapeutic exploration as pharmaceutical modulation of bile salt receptors is evaluated in numerous clinical trials.[9,10] Data from animal experiments indicate that endogenous (i.e., bile salts, BS) or (semi)synthetic ligands (i.e. obeticholic acid, OCA) of the ligand-activated transcription factor Farnesoid X Receptor (FXR) can stimulate liver regeneration (LR) in the context of portal vein embolization or after partial hepatectomy.[11-14] Furthermore, feeding of a cholic acid-enriched diet elicits liver growth in the absence of liver resection.[15] FXR is highly expressed in the small intestine and liver, but also in the adrenal glands and kidneys.[16] FXR plays a central role in maintaining BS homeostasis and, accordingly, acts to limit detrimental effects (e.g. cell death) of BS overload.[16,17] Target genes of FXR include a.o. transporters engaged in uptake (NTCP) and secretion (e.g. BSEP, SLC51A/B) of BS, and genes involved in regulation of bile salt synthesis (e.g. intestinal FGF19/Fgf15 and hepatic SHP (small heterodimer partner)). Endocrine FGF19/15 (fibroblast growth factor) and SHP both target, through distinct routes, the *CYP7A1* gene that encodes the rate-limiting enzyme in the dominating BS synthetic pathway.[16,18]

BS uptake into the ileal enterocyte is the trigger for FXR-mediated transcriptional induction of *FGF19* expression, resulting in enhanced secretion of the enterokine FGF19 (or Fgf15 in rodents) into the portal circulation. The role of Fgf15 in liver regeneration was

first demonstrated by Uriarte et al. who showed the importance of Fxr and Fgf15 in maintaining BS homeostasis in the regenerating liver remnant.[15] Knocking out of either *Fxr* or *Fgf15* led to excessive intrahepatic accumulation of bile salts, increased hepatocellular injury and high mortality in the first 3 days after partial hepatectomy (PH).[11,15] Mouse studies have shown that *Fgf15*$^{-/-}$ mice have increased amounts of hepatic Cyp7a1 at mRNA, protein and functional level.[19] Consequently, liver injury and mortality after PH were negated by intraperitoneal adenoviral-mediated *Fgf15* delivery. Moreover, this study also showed that Fgf15 mediates enhanced liver proliferation following BS feeding. Although signalling actions of BS thus seem required for proper postresectional LR, a tight control of intracellular levels seems indispensable. In addition, Fgf15 seems to exert a direct mitogenic effect on hepatocytes, since knockdown of its receptor fibroblast growth factor receptor 4 (Fgfr4) impaired hepatocyte proliferation after PH.[20] This was probably due to abrogation of Stat3 signalling, which is downstream of Fgfr4, and responsible for induction of *Foxm1b* and subsequent cell cycle progression. In addition to direct mitogenic effects on cultured hepatocytes, FGF19 was reported to enhance growth of cultured cholangiocytes as well.[15]

The aim of our study was to investigate the effect of stimulation of the Fxr/Fgf15 pathway on LR after PH in mice. For this purpose, we used the potent FXR activator OCA, which is approved for treatment of primary biliary cholangitis patients unresponsive to first line therapy and undergoes further clinical evaluation in patients with non-alcoholic steatohepatitis.[9] In the first part of this study, groups of mice were (pre-)treated with vehicle or OCA, and sacrificed within 3 days after PH. It was anticipated that Fxr activation would result in an earlier peak of regenerative indices through direct (i.e., mitogenic) and indirect (i.e., bile salt homeostasis) effects. Unexpectedly, we observed a steep decline in body weight and glycemia after PH in the OCA-treated group. This led us to consider that metabolic effects related to pre-treatment with OCA masked a potential beneficial effect on postresectional LR. We therefore replenished drinking water with sucrose for OCA-treated mice in the second part of the study, whilst mice receiving intraperitoneal injections of FGF19 served as a positive control group.[15]

METHODS

Animal studies

Male C57BL6/J mice (N=36, 11 weeks old) were ordered from Janvier Labs (Le Genest-Saint-Isle, France) and housed in the animal facility of the Université catholique de Louvain (UCL; Brussels, Belgium). The animals were kept under controlled conditions with exposure to a 12-h light/12-h dark cycle and a constant temperature of 20-22°C. Animal

experiments were conducted in accordance with European regulations and FELASA guidelines for humane care for laboratory animals provided by UCL. The study protocol was approved by the university ethics committee (ref nr 2012/UCL/MD/026).

PH model

In the first study, mice were fed standard chow (SAFE diets A03 Augry, France), and were pre-treated for one week with FXR agonist obeticholic acid (OCA dissolved in 0.5% methylcellulose: 10 mg/kg, daily oral gavage, n=18) or vehicle (0.5% methylcellulose, n=18). OCA was generously provided by Intercept Pharmaceuticals Inc, New York, USA. After 7 days of pre-treatment, all mice underwent 70% PH (T=0). PH was performed under anaesthesia with isoflurane and according to the protocol of Mitchell and Willenbring (2008), with the adaptation that instead of an open abdominal procedure, only a small incision below the xyphoid was made through which the liver was mobilized.[21] After resection of the median and lateral lobes and gallbladder, daily oral gavage with OCA and vehicle continued. Two hours prior to sacrifice, all mice were injected in the abdominal cavity with bromodeoxyuridine (BrdU, 50 mg/kg body weight). At the time of sacrifice (24, 48 or 72 hours after PH, n=6 per treatment group and time point), mice were anesthetized via intraperitoneal injection with ketamine/xylazine. The abdominal cavity was opened via midline incision and blood was drawn by portal vein puncture, kept on ice, and serum was prepared and stored at -80°C until further use. Due to technical issues, unfortunately serum was not available for biochemical analyses. Part of the liver ('posterior lobes') and ileum was fixated in 4% paraformaldehyde, with most of the tissues being snap-frozen in liquid nitrogen and stored at -80°C until analysis.

In the second study, mice were pre-treated for one week with OCA (10 mg/kg, daily oral gavage, n=21) or vehicle (0.5% methylcellulose, n=13). As positive control, an additional group of mice underwent daily intraperitoneal injection with FGF19 (1mg/kg body weight in sterile PBS, n=13). Recombinant FGF19 was kindly provided by Genentech, San Francisco, USA. All mice underwent PH after 7 days pre-treatment (n=8/16 per group) or were sacrificed (n=5 per group) to obtain baseline measures. Half of the OCA-treated mice received water supplemented with sucrose (42 g/L) *ad libitum*, to prevent post-PH metabolic derangements. Two days after PH, i.e., around the peak of hepatocyte proliferation, all mice were sacrificed by exsanguination. Tissue and blood were processed in the aforementioned manner.

Liver mass recovery

The rate of liver mass recovery was estimated using the following formula:

$$\textit{Liver mass recovery}\ (\%) = 100 \times \frac{M_s}{M_t}$$

where M_s is the liver weight at sacrifice, and M_t is the total liver mass before PH (estimated by dividing the mass of resected segments by 0.7).

Immunohistochemistry

Hepatocyte proliferation was assessed via Ki67 immunohistochemical staining on serial tissue sections of 4 µm thickness. Mouse monoclonal antibody against Ki67 (1:50; Code No. M7249, Dako, Glostrup, Denmark) was used. Anti-mouse Envision system (Dako) was used for secondary detection. For visualization, the 3,3'-Diaminobenzidine (DAB) Substrate-Chromogen System (Dako) was used. Nuclei were counterstained with hematoxylin.

The proliferative index (%) was determined for both Ki67 and BrdU by dividing the amount of Ki67 or BrdU positive hepatocyte nuclei by the total number of hepatocyte nuclei in five random high-power (40x) fields. In addition, mitotic figures were counted in seven random high-power (20x) fields.

Total bile salt assay

A 5% homogenate of liver tissue was made by homogenizing ca. 50 mg of tissue in 1 mL of 75% ethanol by means of a Mini-Beadbeater (Biospec Products, Bartlesville, USA). Thereafter, BS were extracted by incubating the homogenates for 2 hours at 50°C.[22] Supernatant was collected after centrifugation (10 min., 20620*g* at 4°C). The amount of BS present in serum or liver extract was measured via an enzymatic assay according to the manufacturer's protocol (Total bile acid assay, Diazyme Laboratories, Dresden, Germany). The amount of BS present in liver extract was normalized to liver protein content, as measured by bicinchoninic acid (BCA) assay (Pierce® BCA protein assay kit, Thermo Scientific, Waltham, Massachusetts).

Real-time polymerase chain reaction

Expression levels of genes involved in BS homeostasis, cell cycle regulation and proliferation, cellular stress response, and cytokine regulation were measured in liver and ileum samples via real-time PCR. To study the effect of PH, samples of the quiescent liver (Resected lobes, T=0) were included. RNA was isolated from the resected liver lobe (T=0) and ileal tissue (T=24), and the regenerated lobe at sacrifice (T=48) with TRI reagent solution according to the manufacturer's protocol (Sigma-Aldrich, St. Louis, Missouri). The concentration and purity of RNA in the samples were determined by measuring absorbance with the NanoDrop 1000A spectrophotometer (Thermo Scientific). After RNA was treated with DNAse (Promega, Madison, Wisconsin), efficiency of DNAse treatment was verified by PCR using primers for an intron-less gene. Next, 750 ng total RNA was reverse transcribed to form cDNA according to the manufacturer's protocol (SensiFAST™ cDNA

Synthesis Kit, Bioline, Luckenwalde, Germany). Real-time PCR analyses were performed according to the manufacturer's protocol (SensiMix™ SYBR® & Fluorescein Kit, Bioline) with a MyiQ Single-Color Real-Time PCR detection system (Bio-Rad, Veenendaal, the Netherlands). The reaction mixture contained 2 µL diluted cDNA sample (corresponding to 7.5 ng total RNA) in a total volume of 10 µL. LinRegPCR software was used to calculate relative expression values.[23] Results were normalized using *Rplp0* as a reference gene. Data are expressed relative to the median of the control group at baseline (T=0).

Statistical analysis

Data were statistically analysed using IBM SPSS Statistics version 24 for Microsoft Windows®. Non-normal distribution was assumed, and groups were compared using the Mann-Whitney U test. In case of more than two groups, the Kruskal-Wallis test was applied. If the Kruskal-Wallis test showed significance ($p \leq 0.05$), post-hoc Mann-Whitney U tests were performed within groups with Bonferroni-Holm correction for multiple comparisons. *P*-values ≤0.05 were considered statistically significant. For visual purposes, data on body weight and glycemia in graphs is depicted as mean ± SEM. All experimental data on gene expression and biochemical analyses are graphically presented as median with interquartile range.

RESULTS

OCA pre-treatment alters intestinal and hepatic Fxr target gene expression

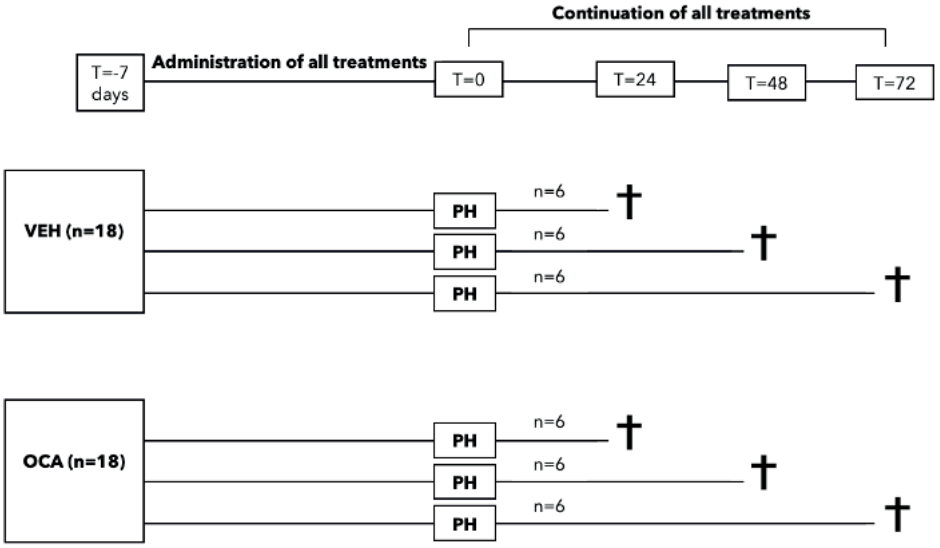
To test the hypothesis that FXR activation augments LR after PH, we examined the effect of OCA on liver regeneration at 24, 48 and 72 hours after PH (Figure 1). Prior to PH, mice received daily OCA gavage for 7 days.

Effectiveness of OCA pre-treatment was inferred from elevated ileal expression of Fxr target genes *Fgf15* and *Slc51b* ($p<0.010$) (Figure 2A, B). Moreover, there was altered expression of Fxr target genes, *viz. Cyp8b1* (-3.3-fold; $p=0.002$) and *Bsep* ($p=0.004$) in the liver (Figure 2C, D). Expression of hepatic *Fxr* per se was not affected by OCA treatment (Figure 2E).

OCA has no effect on functional liver generation parameters

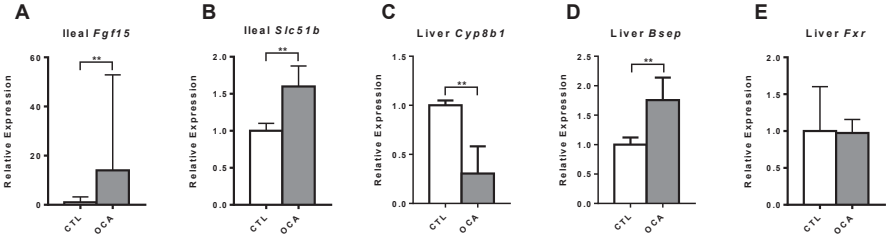
OCA had no effect on body weight (Figure 3A) or glycaemia course (data not shown) during the pre-treatment period. PH resulted in a transient drop in body weight in the first 2 days after surgery, with body weight returning to pre-surgical values at T=72h in the control group. Of note, OCA-treated mice continued to lose weight after post-operative day 2.

Figure 1. Study design



Mice (n=6 mice per group) were pre-treated for 7 days by daily administration of OCA or vehicle, before undergoing 70% PH (T=0) with continuation of treatments. Mice were sacrificed at 24, 48 and 72 hours after PH while treatments continued. VEH, vehicle; OCA, obeticholic acid; PH, partial hepatectomy

Figure 2. Effect of pre-treatment with OCA on expression of Fxr target genes

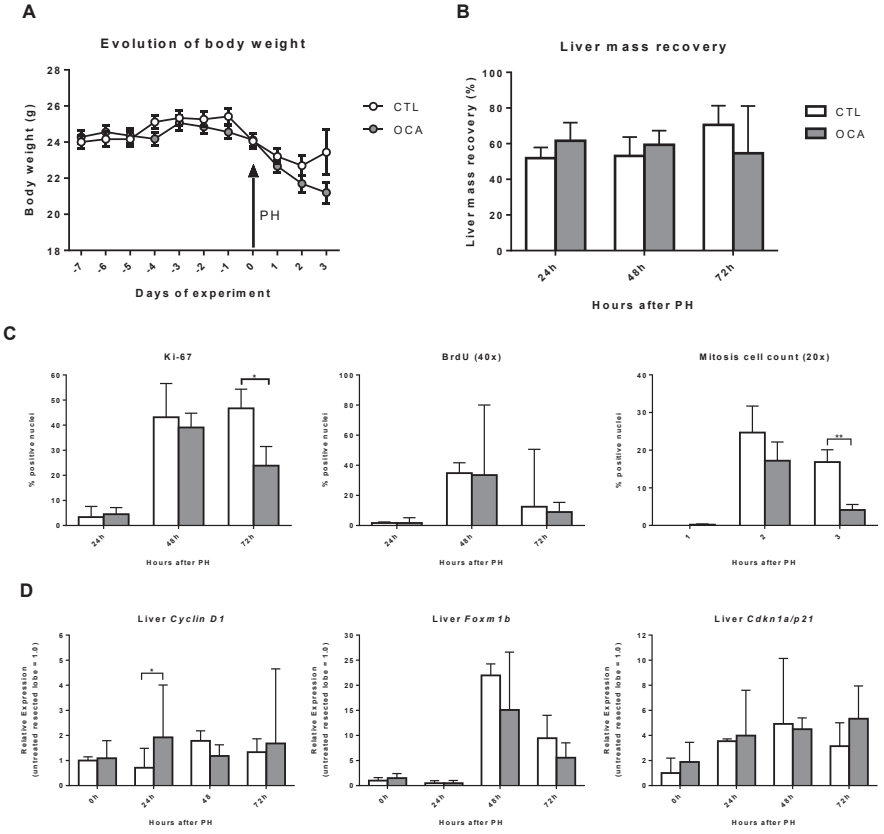


Mice (n=6 mice per group) were pre-treated for 7 days by daily administration of OCA or vehicle, before undergoing 70% PH. Transcripts were analysed in the terminal ileum of mice sacrificed 24 hrs after PH **(A-C)**, and the liver (segments resected at T=0, **D, E**). Values are expressed relative to the median expression in the control group. **p<0.01; OCA, obeticholic acid; Fgf, fibroblast growth factor; Slc51b, organic solute transporter beta; Cyp8b1, sterol 12-α-hydroxylase; Bsep, bile salt export pump; Fxr, farnesoid x receptor

Liver mass recovery calculations showed no differences between OCA-treated animals and control group at each of the studied time points after PH (Figure 3B). DNA synthesis has been reported to increase at 32 hours post-hepatectomy in mice, with an initial peak reflecting hepatocyte division at 36-40 hours.[24] Here, we observed a peak in nuclear Ki67 staining in hepatocytes, BrdU incorporation and mitotic events at 48 hours after PH (Figure 3C). OCA treatment did not result in an earlier peak and had no effect on the percentage of Ki67+ hepatocytes, BrdU incorporation or the number of mitotic figures at T=48h. In fact, the number of mitotic figures and Ki67+ nuclei at T=72h was decreased in OCA-treated mice (both $p<0.05$). Above observations are not in support of our hypothesis.

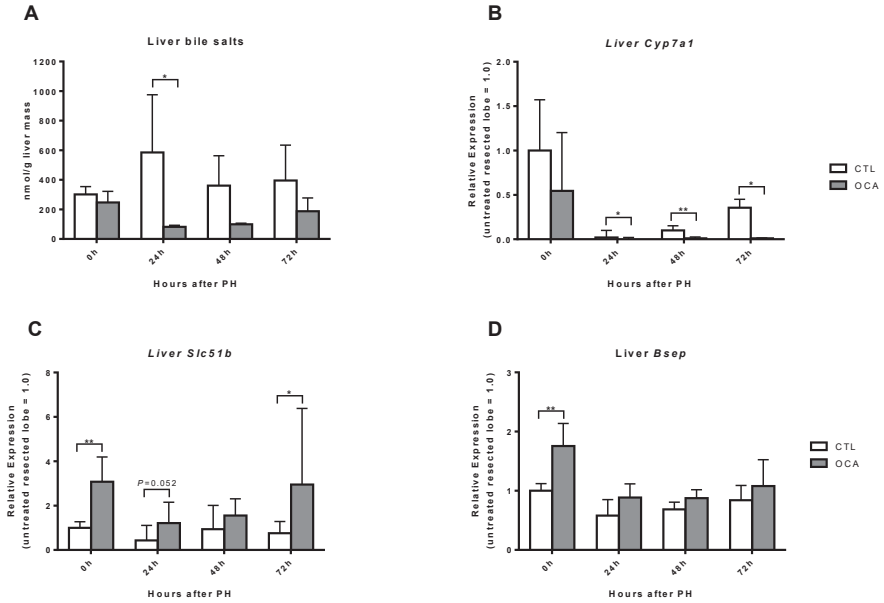
Next, we determined whether PH elicited expected changes in expression of key genes in cell cycle progression. Cyclin D1, pivotal for progression of hepatocytes through G1 phase, starts increasing at approximately 30 hours after PH (prior to peak DNA synthesis).[25-27] Expression of Cyclin D1 was increased in the OCA-treated animals at 24 hours after PH in comparison to the control group (Figure 3D). This did not translate into functional changes later in the cell cycle or regenerative course (i.e., liver mass recovery, mitotic figures, and %Ki67^{+} hepatocytes). PH resulted in strong induction of Foxm1b from T=48h onwards. The extent of upregulation was similar in both groups at T=48h and T=72h, despite Foxm1b being an Fxr target gene. Liver expression of Cdkn1a, involved in G1 phase cell cycle arrest, was equally upregulated in both groups after PH.

Figure 3. Effect of pre-and posttreatment with OCA on functional parameters, proliferative measures and expression of Fxr target genes



Mice (n=6 mice per group) were pre-treated for 7 days by daily administration of OCA or vehicle, before undergoing 70% PH. Mice were sacrificed at 24, 48 and 72 hours after PH while treatments continued. Body weight was recorded daily from 7 days before surgery to sacrifice **(A)**. Regeneration after PH was assessed by recovery of liver mass **(B)** and immunohistochemical analysis of hepatocyte proliferation **(C)**. Data are presented as mean with SEM. Transcripts were analysed in the liver **(D)**. Values are expressed relative to the median expression in the control group. *p<0.05; **p<0.01; OCA, obeticholic acid; PH, partial hepatectomy; BrdU, bromodeoxyuridine; Ccn, cyclin; Fox, forkhead box; Cdkn, cyclin dependent kinase inhibitor

Figure 4. Effect of pre-and posttreatment with OCA or vehicle on bile salt levels and activation of hepatic and ileal Fxr-Fgf15 pathways



Mice (n=6 mice per group) were pre-treated for 7 days by daily administration of OCA or vehicle, before undergoing 70% PH. Mice were sacrificed at 24, 48 and 72 hours after PH while treatments continued. Total bile salts levels in the liver **(A)** were analysed. Data are presented as mean with SEM. Transcripts **(B-D)** were analysed in the liver. Values are expressed relative to the median expression in the control group. *p<0.05; **p<0.01; OCA, obeticholic acid; PH, partial hepatectomy; Cyp7a1, cholesterol 7α-hydroxylase; Slc51b, organic solute transporter beta; Bsep, bile salt export pump

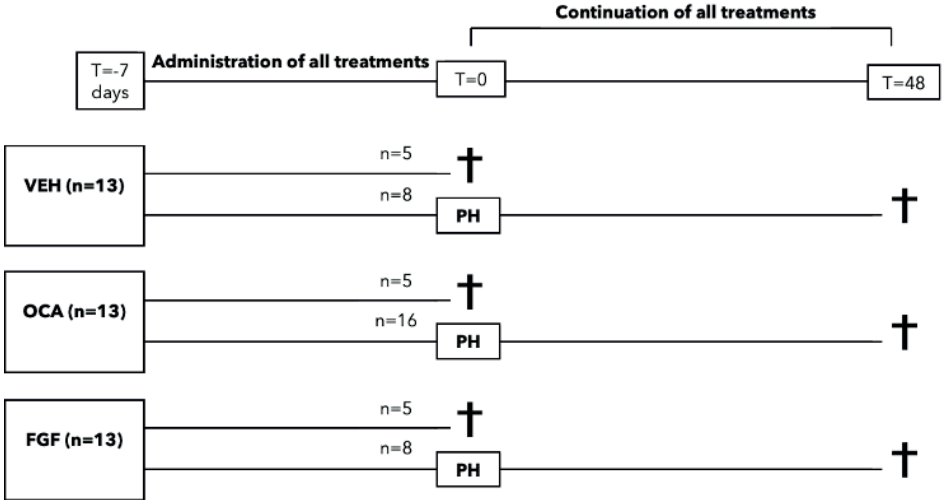
OCA reduces BS levels in the regenerating liver

Maintenance of BS homeostasis is essential for normal progression of liver regeneration after PH. To see whether lack of effects of OCA on liver regrowth was due to disturbed BS homeostasis, we determined hepatic BS content in the quiescent and regenerating liver. OCA led to decreased BS content in the remnant liver, which reached significance at T=24h (Figure 4A). Note that there was substantial variation in remnant liver BS content in control mice in particular. PH resulted in marked repression of Cyp7a1, with superimposed downregulation by OCA at all time points after PH (Figure 4B). In addition to reduced BS synthesis, enhanced basolateral efflux of BS via upregulated expression of Slc51b may have contributed to reduced BS content in the OCA-treated group (Figure 4C). Bsep was upregulated by OCA solely before PH (Figure 4D).

Post-PH metabolic derangements (no body weight recovery, reduced serum glucose) and poorer well-being (scored by for example rough hair coat, squinted eyes, hunched walking) were observed in OCA-treated animals, and this may have masked effects of OCA on liver regeneration after PH. At histological examination of the liver, there were no signs of injury, cholestasis, or necrosis.

In the second part of this study, half of the hepatectomized mice given OCA therefore received water supplemented with sucrose. To see whether liver regeneration could be enhanced under our experimental conditions, an additional group of mice was treated with recombinant FGF19, which was previously shown to stimulate hepatocellular proliferation.[15] Per group, 5 mice were sacrificed after pre-treatment for one week without undergoing PH, to determine baseline effects of OCA and FGF19 pre-treatment. The other animals were subjected to 70% PH and sacrificed 48 hrs later (Figure 5).

Figure 5. Study design follow-up study



Mice were pre-treated for 7 days by daily administration of OCA, FGF19 or vehicle, before sacrifice (n=5) of 70% PH with continuation of treatments (n=8-16). Mice that were subjected to 70% PH were sacrificed 48 hrs later. VEH, vehicle; OCA, obeticholic acid; FGF, fibroblast growth factor; PH, partial hepatectomy

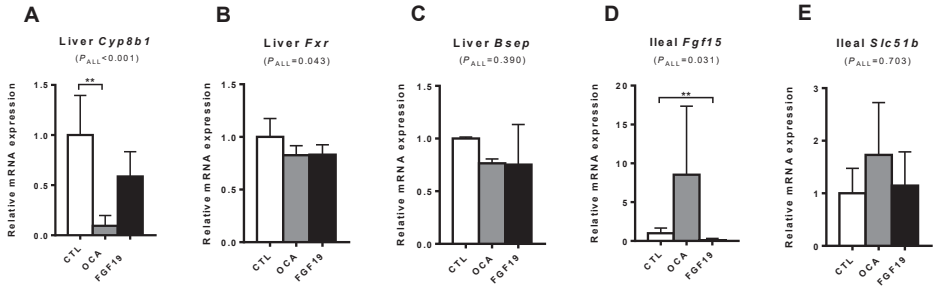
Effects of pre-treatment with OCA and FGF19

In mice sacrificed without undergoing PH, the hepatic Fxr pathway was activated as shown by downregulation of Cyp8b1 (Figure 6A). This study confirmed an unchanged hepatic Fxr expression after Fxr agonism, also without PH (Figure 6B).[28] In contrast to the first part of the study where baseline effects were derived from resected segments, thus, with potential superimposed effects of liver mobilization and surgical manipulation, OCA pre-treatment had no effect on Bsep (Figure 6C) and Slc51b expression (data not shown).[29] At the level of the ileum, OCA tended to induce expression of Fgf15 (p=0.151), whilst Slc51b expression was unaltered (Figure 6D, E).

Effectiveness of FGF19 treatment was tested by studying expression of Cyp7a1, which is repressed as consequence of binding of FGF19 to its hepatic receptor (Fgfr4). Unexpectedly, Cyp7a1 levels showed a strong trend but were not significantly affected by FGF19

treatment (p=0.053, Figure 9C). In contrast, ileal expression of the bile salt-regulated gene Fgf15 was reduced by FGF19 (Figure 6D), which can be interpreted as a secondary consequence of FGF19-mediated repression of bile salt synthesis, with a smaller supply of bile salts reaching the small intestine. FGF19 did not affect hepatic expression of Cyp8b1 and Bsep (Figure 6A, C). Hepatocyte receptors Fgfr4 and Klb, for which FGF19/Fgf15 is a ligand, were expressed to the same extent in all groups (Supplemental figure 1).

Figure 6. Effect of pre-treatment with OCA, FGF19 or vehicle on activation of hepatic and ileal Fxr-Fgf15 pathways



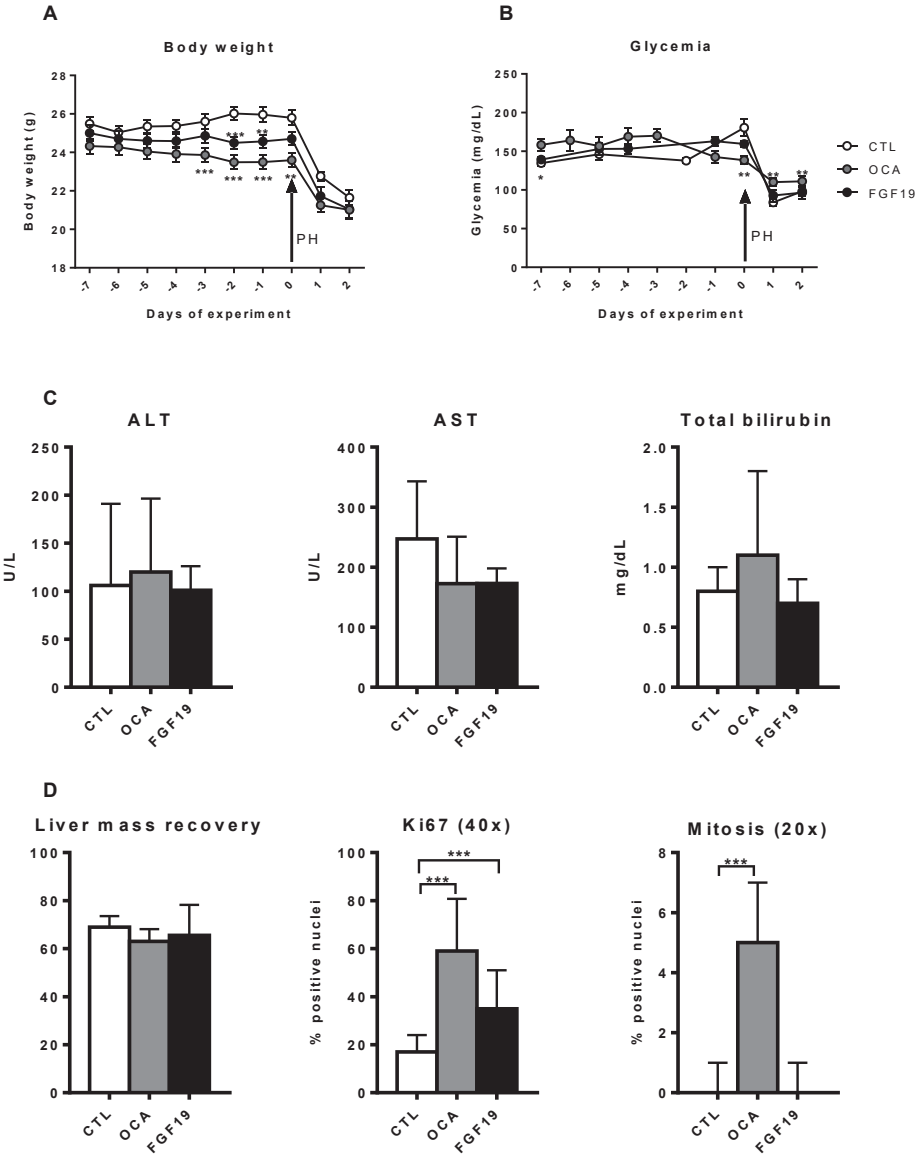
Mice (n=5 mice per group) were pre-treated for 7 days by daily administration of OCA, FGF19 or vehicle, before sacrifice. Transcripts were analysed in the liver **(A-C)**, and the terminal ileum **(D, E)**. Values are expressed relative to the median expression in the control group. **p<0.01; OCA, obeticholic acid; FGF, fibroblast growth factor; Cyp8b1, sterol 12-α-hydroxylase; Fxr, farnesoid X receptor; Bsep, bile salt export pump; Slc51b, organic solute transporter beta

We next determined effects of OCA and FGF19 on liver regeneration at 48 hrs after PH. Mice treated with OCA and receiving sucrose-supplemented water in the post-PH course, were indistinguishable on all examined parameters (body weight, glucose, well-being, serum biochemistry, bile salt levels, gene expression) from OCA-treated mice receiving plain water. We therefore decided to merge data of these groups into a single group of n=16 (Figure 7A, B). Levels of circulating liver enzymes and bilirubin at 48 hrs after resection were similar between groups (Figure 7C), showing a 1.5-2-fold increase in all groups compared to baseline values (not shown). Liver mass recovery after PH was comparable between groups (Figure 7D), whilst the number of hepatocyte nuclei positive for Ki67 and mitotic figures were significantly increased in the OCA-treated animals (Figure 7D).

Effect of OCA and FGF19 on cell cycle progression after PH

Increased proliferation after OCA treatment was not corroborated by upregulation of Fxr target gene Foxm1b (Figure 8A) and its downstream target gene Cdc25b (Figure 8B).[11] Expression of cyclins Ccnd1 and Ccne1 was not increased compared to control animals (Figure 8C, D). In contrast, an upregulation of pivotal regulator of cell cycle progression Ccna2 was detected in animals treated with OCA (Figure 8E).

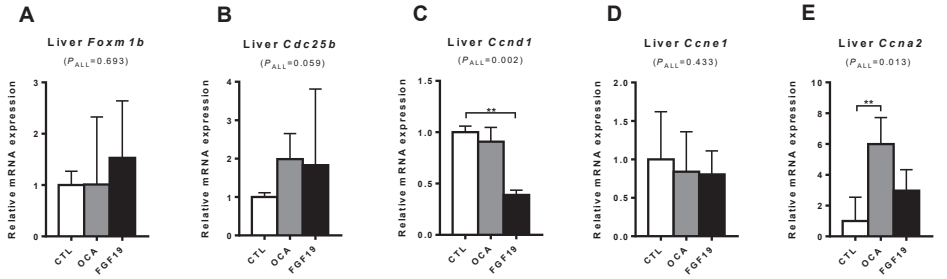
Figure 7. Effect of pre -and posttreatment with OCA, FGF19 or vehicle on functional parameters, liver biochemistry and proliferative measures



Mice (n=8-16 mice per group) were pre-treated for 7 days by daily administration of OCA, FGF19 or vehicle, before undergoing 70% PH. Mice were sacrificed at 48 hours after PH while treatments continued. Body weight **(A)** and glycemia levels **(B)** were recorded daily from 7 days before surgery to sacrifice. Liver enzymes and total bilirubin were determined in serum at exsanguination **(C)**. Regeneration after PH was assessed by recovery of liver mass and immunohistochemical analysis of hepatocyte proliferation **(D)**. ***p<0.001; **p<0.01; OCA, obeticholic acid; FGF, fibroblast growth factor; PH, partial hepatectomy; ALT, alanine aminotransferase; AST, aspartate aminotransferase

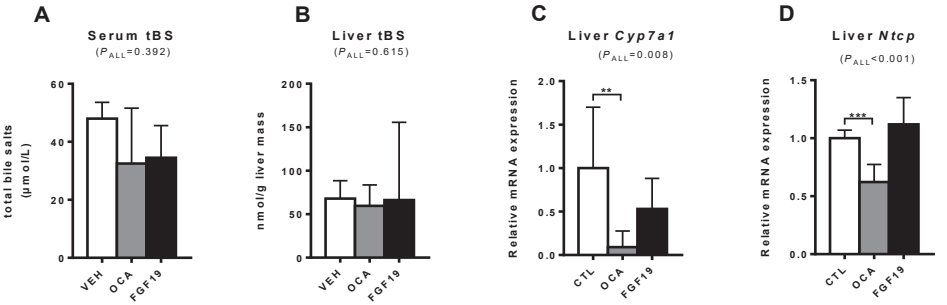
In animals treated with FGF19, Foxm1b did not change after PH, whilst expression of Ccnd1 was decreased. No significant upregulation of cell cycle progression regulator Cdc25b was seen compared to the control group, which was similar for Ccne1 and Ccna2.

Figure 8. Effect of pre-and posttreatment with OCA, FGF19 or vehicle on expression of Fxr target gene and cyclins playing a key role in cell cycle progression



Mice (n=8-16 mice per group) were pre-treated for 7 days by daily administration of OCA, FGF19 or vehicle, before undergoing 70% PH. Mice were sacrificed at 48 hours after PH while treatments continued. Transcripts **(A-E)** were analysed in the liver (segments resected at T=48). Values are expressed relative to the median expression in the control group. **p<0.01; OCA, obeticholic acid; FGF, fibroblast growth factor; Fox, forkhead box; Cdc, cell division cycle; Ccn, cyclins

Figure 9. Effect of pre- and posttreatment with OCA, FGF19 or vehicle on bile salt levels and Fxr target genes involved in bile salt synthesis and uptake



Mice (n=8-16 mice per group) were pre-treated for 7 days by daily administration of OCA, FGF19 or vehicle, before undergoing 70% PH. Mice were sacrificed at 48 hours after PH while treatments continued. Total serum and liver bile salts levels **(A, B)** and transcripts **(C, D)** were analysed in the liver. Values are expressed relative to the median expression in the control group. ***p<0.001; **p<0.01; VEH/Ctl, control; OCA, obeticholic acid; FGF, fibroblast growth factor; tBS, total bile salt(s); Cyp7a1, cholesterol 7α-hydroxylase; Ntcp, sodium-taurocholate co-transporting polypeptide

Serum and hepatic bile salt content was not different between groups (Figure 9A, B), despite a reduced expression of Cyp7a1 in animals treated with OCA (Figure 9C). The decrease in Cyp7a1 expression was not seen in the animals receiving FGF19, suggesting that co-activation of the ileal and hepatic axis is important for optimal repression of Cyp7a1. The time interval between administration of FGF19 and liver harvesting might also be a reason for the lack of effect of FGF19 on Cyp7a1 expression. Animals were sacrificed 0.5-2.5 hrs after the last administration of OCA/FGF19, whilst in vitro studies in HepG2 cells

(data not shown) indicate that it takes at least 2 hrs before CYP7A1 mRNA is significantly reduced by FGF19. Expression of Ntcp was reduced in mice receiving OCA (Figure 9D).

DISCUSSION

Bile salts have emerged as essential signalling molecules in liver regeneration after PH. Data from animal experiments indicate that endogenous ligands (i.e., bile salts) can stimulate liver regeneration and prevent liver injury after partial hepatectomy via the hepatic FXR and ileal Fxr-Fgf15 axis. Our aim was to investigate whether exogenous activation of the Fxr pathway with the semi-synthetic bile acid OCA (a.k.a. INT747) could stimulate postresectional liver regeneration in mice. We observed inconsistent effects of OCA on regenerative indices in hepatectomized mice. Although responses of Cyp7a1, Cyp8b1 and other Fxr targets implied general effectiveness of OCA treatment, no effect could be detected on liver mass recovery after PH (Figure 3B, 7D). Moreover, OCA had no effect on or increased the number of $Ki67^{+}$ hepatocytes and mitotic figures at 48 hrs after PH (Figure 3C, 7D). Hepatic bile salt content did not improve around the peak of hepatocyte proliferation at 48 hrs after PH (Figure 4A, 9B). Besides, welfare of the animals was decreased in OCA-treated animals after PH.

Although we did not detect functional effects on liver regeneration after treatment with OCA, activation of hepatic Fxr was implicated by repression of Cyp8b1 in mice pre-treated with OCA in both studies. Simultaneously, activation of ileal Fxr was demonstrated by increased Fgf15 after pre-treatment with OCA, and decreased Cyp7a1 expression after PH in both studies. Treatment with OCA enhanced these Fxr-mediated effects. However, this had no consistent effects on regenerative indices other than liver mass recovery, which was not influenced by OCA in both studies. Although liver mass regrowth is not an indicator for functional liver recovery per se, it is one of the most used parameters to capture this complex cascade of events. This suggests that liver regeneration was already progressing optimally after vehicle treatment and Fxr agonism did not further benefit liver regeneration after 70% PH. It must be highlighted, though, that liver mass estimation can be easily influenced by for example surgical procedure, inter-surgeon variability and hepatic oedema.

In these experiments we studied experimental mouse models with uncompromised liver function. Compromised liver quality such as cirrhotic or cholestatic livers, and extended liver resection, are important factors for leaving patients ineligible for PH in clinical practice. Moreover, upon surgery PLF and mortality are increased in these patient groups. After PH, it is suggested that a relative hepatic overload of potentially toxic bile salts is

one of the causative factors for PLF.[15] Cholestasis decreases hepatic regenerative capacity.[30] Comparable rodent models with disrupted bile salt homeostasis and decreased liver regeneration comprise an extensive (90%) hepatectomy,[15] and bile duct ligation with consequent cholestasis.[31,32] Rodents receiving cholestyramine or pre-operative bile diversion to deplete the whole-body bile salt pool, also showed decreased liver regrowth after PH, emphasizing the necessity of maintaining bile salt signalling and/or homeostasis.[11] A beneficial effect of Fxr agonism was already shown by Chen et al.,[33] where Fxr agonism alleviated the age-related liver regeneration defect, highlighting Fxr as a potential target for promoting liver regeneration in older patients. Moreover, OCA treatment may increase the efficacy of PVE and, thereby, resectability. Earlier we showed that OCA accelerated liver regeneration after experimental portal vein embolization, in terms of liver volume, liver function and proliferation.[14]

The beneficial metabolic effects of FXR agonists, OCA in particular, and FGF19 have been widely studied in many human and experimental settings. Substantial numbers of (selective) FXR agonists have been tested in rodents to study proliferative and metabolic responses.[34,35] Improved serum enzymes and reduced steatosis after PH were described upon treatment with synthetic FXR agonist GW4064, but only limited proliferative effects were seen.[11,36] Perioperative oral gavage with alisol B 23-acetate (AB23A) resulted in upregulation of Fxr-dependent proliferative genes, amelioration of liver injury, and decreased bile salt synthesis and hepatic bile salt content, after 70% in a non-compromised mouse model.[37] Earlier pre-clinical studies demonstrated that OCA treatment resulted in improvement of hepatic non-alcoholic steatohepatitis (NASH) and cirrhosis on histological level (fibrosis, hepatocellular ballooning, steatosis, and lobular inflammation),[9,38,39] portal hypertension,[40] increased metabolic rate[41] and atherosclerosis by increasing faecal cholesterol excretion[42]. Recent studies focusing on intestinal benefits described preservation of intestinal mucosal wall integrity, attenuation of intestinal inflammation and reduced bacterial translocation after OCA treatment in rat models on cholestasis and intestinal ischemia-reperfusion.[43,44] Also, the effects of (long-term) Fxr activation on carcinogenesis are controversial,[45] with prevention of hepatic and renal tumour formation after Fxr agonism and hepatic tumorigenesis in Fxr KO mice on one side,[46-48] and high FXR expression related to high tumour aggressiveness and poor prognosis on the other side.[49-52] Furthermore, generalised pruritus occurred more frequently after OCA treatment, but the underlying mechanism is poorly understood.[9,53] In our studies, we found decreased serum glucose levels before and after PH, and significant weight loss after treatment with OCA. These metabolic effects were comparable to data in rodent and human studies,[9] however seemed to be accompanied by a decreased score on our welfare assessment. It appeared that in the perioperative setting, mice were too vulnerable to cope with these metabolic changes which may have affected liver regrowth as well. Many of the metabolic (side)

effects of OCA may be explained by the large number of known but yet uncharacterized Fxr target genes.[54]

In the second study we added an experimental group that underwent intraperitoneal injection of FGF19. In the study of Uriarte et al., adenoviral-mediated expression of FGF19 abrogated the diminished liver regeneration in Fgf15 KO mice. In the liver with a compromised background (*viz.* impaired liver regeneration due to acetaminophen poisoning and partial hepatectomy in aged mice), Alvarez et al. [55] showed that the chimeric FGF19/apolipoprotein A-I molecule Fibapo attenuated liver injury, boosted regeneration as seen by potentiated cell growth-related pathways and increased functional liver mass, and improved survival. In another experiment from the same research group, Fibapo reversed elevated hepatic Pparγ2 expression in Fgf15-/- mice fed a high fat diet. Furthermore, Fibapo reduced liver bile salt and lipid accumulation, and resulted in increased survival and improved regeneration after PH.[56]

In our study, we found that treatment with FGF19 did not stimulate postresectional liver regeneration in mice. Although reduction of expression of ileal bile salt-regulated genes Fgf15 and Slc51b indicating FGF19-mediated repression of bile salt synthesis was seen after pre-treatment, FGF19 did not affect hepatic expression of Cyp8b1 and Bsep (Figure 6). Also, hepatocyte receptors Fgfr4 and Klb, for which FGF19/Fgf15 is a ligand, were expressed to the same extent in all groups (Supplemental figure 1).

After PH, Foxm1b was significantly upregulated but FGF19 had no stimulatory effect on cell cycle progression regulators Cdc25b, Ccne1, Ccna2 and Ccnd1 (Figure 8). In addition, liver mass recovery or mitotic figures at 48 hrs after PH (Figure 7D) were not affected by treatment with FGF19, although the number of Ki67+ hepatocytes was increased. Hepatic bile salt content did not improve around the peak of proliferation at 48 hrs after PH (Figure 9B).

Csanaky et al. demonstrated that after PH, hepatocytes were protected from bile salt toxicity by increased canalicular and basolateral bile salt secretion resulting in increased serum bile salt levels, whereas total hepatic bile salt content tended to increase but was not significantly influenced by PH.[57] Although Bsep gene expression stayed the same as confirmed in our study, protein expression doubled. Moreover, they found a change in hepatic bile salt content in favour of unconjugated bile salts. In our study, we observed unaltered serum bile salts after partial hepatectomy in all groups. Furthermore, despite upregulation of Slc51b and repression of Cyp7a1 and Cyp8b1, hepatic bile salt content did not decrease in OCA-treated animals. Exploring bile salt composition in serum and liver would be interesting to detect a possible shift in toxicity.

Collectively, our results show that despite the activation of hepatic and ileal Fxr as shown by induction of its target genes, treatment with OCA does not result in accelerated liver regeneration after PH. Moreover, serum and liver bile salt content were not influenced by treatment before and after PH. It would be interesting to learn what would be the effect of administering OCA in the post-PH phase only. In such set-up, groups of animals would have the same metabolic starting point. We speculate that bile salt homeostasis is already optimally maintained for proper progression of liver regeneration/repair after PH. In the experimental or clinical setting of compromised bile salt homeostasis/signalling prior to partial hepatectomy, e.g., due to external bile diversion or cholestasis, FXR agonism may be of benefit.

REFERENCES

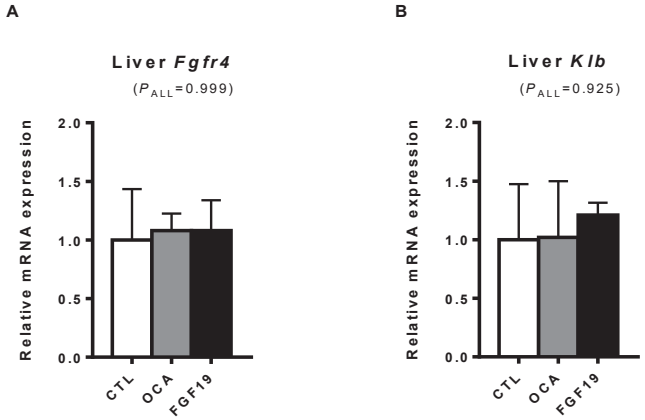
1. van den Broek MA, Olde Damink SW, Dejong CH, et al. Liver failure after partial hepatic resection: definition, pathophysiology, risk factors and treatment. *Liver int* 2008; **28**:767-780.
2. Schindl MJ, Millar AM, Redhead DN, et al. The adaptive response of the reticuloendothelial system to major liver resection in humans. *Ann Surg* 2006; **243**:507-514.
3. van Lienden KP, van den Esschert JW, de Graaf W, et al. Portal vein embolization before liver resection: a systematic review. *Cardiovasc Intervent Radiol* 2013; **36**:25-34.
4. Schnitzbauer AA, Lang SA, Goessmann H, et al. Right portal vein ligation combined with in situ splitting induces rapid left lateral liver lobe hypertrophy enabling 2-staged extended right hepatic resection in small-for-size settings. *Ann Surg* 2012; **255**:405-414.
5. Schadde E, Malago M, Hernandez-Alejandro R, et al. Monosegment ALPPS hepatectomy: extending resectability by rapid hypertrophy. *Surgery* 2015; **157**:676-689.
6. Fausto N, Campbell JS, Riehle KJ. Liver regeneration. *J Hepatol* 2012; **57**:692-694.
7. Michalopoulos GK. Principles of liver regeneration and growth homeostasis. *Compr Physiol* 2013; **3**:485-513.
8. Hu J, Srivastava K, Wieland M, et al. Endothelial cell-derived angiopoietin-2 controls liver regeneration as a spatiotemporal rheostat. *Science* 2014; **343**:416-419.
9. Neuschwander-Tetri BA, Loomba R, Sanyal AJ, et al. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. *Lancet* 2015; **385**:956-965.
10. Venetsanaki V, Karabouta Z, Polyzos SA. Farnesoid X nuclear receptor agonists for the treatment of nonalcoholic steatohepatitis. *Eur J Pharmacol* 2019; **863**:172661.
11. Huang W, Ma K, Zhang J, et al. Nuclear receptor-dependent bile acid signalling is required for normal liver regeneration. *Science* 2006; **312**:233-236.
12. Zhang L, Wang YD, Chen WD, et al. Promotion of liver regeneration/repair by farnesoid X receptor in both liver and intestine in mice. *Hepatology* 2012; **56**:2336-2343.
13. van de Laarschot LF, Jansen PL, Schaap FG, Olde Damink SW. The role of bile salts in liver regeneration. *Hepatol Int* 2016; **10**:733-740.
14. Olthof PB, Huisman F, Schaap FG, et al. Effect of obeticholic acid on liver regeneration following portal vein embolization in an experimental model. *Br J Surg* 2017; **104**:590-599.
15. Uriarte I, Fernandez-Barrena MG, Monte MJ, et al. Identification of fibroblast growth factor 15 as a novel mediator of liver regeneration and its application in the prevention of post-resection liver failure in mice. *Gut* 2013; **62**:899-910.
16. Jonker JW, Liddle C, Downes M. FXR and PXR: potential therapeutic targets in cholestasis. *J Steroid Biochem Mol Biol* 2012; **130**:147-158.
17. Uriarte I, Latasa MU, Carotti S, et al. Ileal FGF15 contributes to fibrosis-associated hepatocellular carcinoma development. *Int J Cancer* 2015; **136**:2469-2475.
18. Schaap FG, Trauner M, Jansen PL. Bile acid receptors as targets for drug development. *Nat Rev Gastroenterol Hepatol* 2014; **11**:55-67.
19. Inagaki T, Choi M, Moschetta A, et al. Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. *Cell Metab* 2005; **2**:217-225.
20. Padrissa-Altes S, Bachofner M, Bogorad RL, et al. Control of hepatocyte proliferation and survival by Fgf receptors is essential for liver regeneration in mice. *Gut* 2015; **64**:1444-1453.
21. Mitchell C, Willenbring H. A reproducible and well-tolerated method for 2/3 partial hepatectomy in mice. *Nat Protoc* 2008; **3**:1167-1170.

22. Modica S, Murzilli S, Moschetta A. Characterizing Bile Acid and Lipid Metabolism in the Liver and Gastrointestinal Tract of Mice. *Curr Protoc Mouse Biol* 2011; **1**:289-321.
23. Ramakers C, Ruijter JM, Deprez RH, Moorman AF. Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. *Neurosci Lett* 2003; **339**:62-66.
24. Weglarz TC, Sandgren EP. Timing of hepatocyte entry into DNA synthesis after partial hepatectomy is cell autonomous. *Proc Natl Acad Sci U S A* 2000; **97**:12595-12600.
25. Rickheim DG, Nelsen CJ, Fassett JT, Timchenko NA, Hansen LK, Albrecht JH. Differential regulation of cyclins D1 and D3 in hepatocyte proliferation. *Hepatology* 2002; **36**:30-38.
26. Sun W, Lee DK, Lee CC, Kim K. Differential expression of D-type G1 cyclins during mouse development and liver regeneration in vivo. *Mol Reprod Dev* 1996; **43**:414-420.
27. Albrecht JH, Hu MY, Cerra FB. Distinct patterns of cyclin D1 regulation in models of liver regeneration and human liver. *Biochem Biophys Res Commun* 1995; **209**:648-655.
28. Zhang JH, Nolan JD, Kennie SL, et al. Potent stimulation of fibroblast growth factor 19 expression in the human ileum by bile acids. *Am J Physiol Gastrointest Liver Physiol* 2013; **304**:940-948.
29. Ballatori N, Li N, Fang F, Boyer JL, Christian WV, Hammond CL. OST alpha-OST beta: a key membrane transporter of bile acids and conjugated steroids. *Front Biosci (Landmark Ed)* 2009; **14**:2829-2844.
30. Yokoyama Y, Nagino M, Nimura Y. Mechanism of impaired hepatic regeneration in cholestatic liver. *J Hepatobiliary Pancreat Surg* 2007; **14**:159-166.
31. Modica S, Petruzzelli M, Bellafante E, et al. Selective activation of nuclear bile acid receptor FXR in the intestine protects mice against cholestasis. *Gastroenterology* 2012; **142**:355-365.
32. van Golen RF, Olthof PB, Lionarons DA, et al. FXR agonist obeticholic acid induces liver growth but exacerbates biliary injury in rats with obstructive cholestasis. *Sci Rep* 2018; **8**:16529.
33. Chen WD, Wang YD, Zhang L, et al. Farnesoid X receptor alleviates age-related proliferation defects in regenerating mouse livers by activating forkhead box m1b transcription. *Hepatology* 2010; **51**:953-962.
34. Abel U, Schluter T, Schulz A, et al. Synthesis and pharmacological validation of a novel series of non-steroidal FXR agonists. *Bioorg Med Chem Lett* 2010; **20**:4911-4917.
35. Verbeke L, Nevens F, Laleman W. Steroidal or non-steroidal FXR agonists - Is that the question? *J Hepatol* 2017; **66**:680-681.
36. Haga S, Yimin, Ozaki M. Relevance of FXR-p62/SQSTM1 pathway for survival and protection of mouse hepatocytes and liver, especially with steatosis. *BMC Gastroenterol* 2017; **17**:9.
37. Meng Q, Chen X, Wang C, et al. Alisol B 23-acetate promotes liver regeneration in mice after partial hepatectomy via activating farnesoid X receptor. *Biochem Pharmacol* 2014; **92**:289-298.
38. Roth JD, Feigh M, Veidal SS, et al. INT-767 improves histopathological features in a diet-induced ob/ob mouse model of biopsy-confirmed non-alcoholic steatohepatitis. *World J Gastroenterol* 2018; **24**:195-210.
39. Verbeke L, Mannaerts I, Schierwagen R, et al. FXR agonist obeticholic acid reduces hepatic inflammation and fibrosis in a rat model of toxic cirrhosis. *Sci Rep* 2016; **6**:33453.
40. Schwabl P, Hambruch E, Seeland BA, et al. The FXR agonist PX20606 ameliorates portal hypertension by targeting vascular remodelling and sinusoidal dysfunction. *J Hepatol* 2017; **66**:724-733.
41. Fu L, John LM, Adams SH, et al. Fibroblast growth factor 19 increases metabolic rate and reverses dietary and leptin-deficient diabetes. *Endocrinology* 2004; **145**:2594-2603.
42. Xu Y, Li F, Zalzala M, et al. Farnesoid X receptor activation increases reverse cholesterol transport by modulating bile acid composition and cholesterol absorption in mice. *Hepatology* 2016; **64**:1072-1085.
43. Verbeke L, Farre R, Verbinnen B, et al. The FXR agonist obeticholic acid prevents gut barrier dysfunction and bacterial translocation in cholestatic rats. *Am J Pathol* 2015; **185**:409-419.

44. Ceulemans LJ, Verbeke L, Decuypere JP, et al. Farnesoid X Receptor Activation Attenuates Intestinal Ischemia Reperfusion Injury in Rats. *PLoS One* 2017; **12**:0169331.
45. Schaap FG, Jansen PLM, Olde Damink SWM. Chronic elevation of plasma fibroblast growth factor 19 in long-term farnesoid X receptor agonist therapy, a happy marriage or cause for oncological concern? *Hepatology* 2018; **67**:782-784.
46. Cariello M, Peres C, Zerlotin R, et al. Long-term Administration of Nuclear Bile Acid Receptor FXR Agonist Prevents Spontaneous Hepatocarcinogenesis in Abcb4(-/-) Mice. *Sci Rep* 2017; **7**:11203.
47. Fujino T, Sakamaki R, Ito H, et al. Farnesoid X receptor regulates the growth of renal adenocarcinoma cells without affecting that of a normal renal cell-derived cell line. *J Toxicol Sci* 2017; **42**:259-265.
48. Deuschle U, Schuler J, Schulz A, et al. FXR controls the tumor suppressor NDRG2 and FXR agonists reduce liver tumor growth and metastasis in an orthotopic mouse xenograft model. *PLoS One* 2012; **7**:43044.
49. Giaginis C, Tsoukalas N, Alexandrou P, et al. Clinical significance of farnesoid X receptor expression in thyroid neoplasia. *Future Oncol* 2017; **13**:1785-1792.
50. You W, Chen B, Liu X, Xue S, Qin H, Jiang H. Farnesoid X receptor, a novel proto-oncogene in non-small cell lung cancer, promotes tumor growth via directly transactivating CCND1. *Sci Rep* 2017; **7**:591.
51. Hu H, Wu LL, Han T, et al. Correlated high expression of FXR and Sp1 in cancer cells confers a poor prognosis for pancreatic cancer: A study based on TCGA and tissue microarray. *Oncotarget* 2017; **8**:33265-33275.
52. Nicholes K, Guillet S, Tomlinson E, et al. A mouse model of hepatocellular carcinoma: ectopic expression of fibroblast growth factor 19 in skeletal muscle of transgenic mice. *Am J Pathol* 2002; **160**:2295-2307.
53. Kowdley KV, Luketic V, Chapman R, et al. A randomized trial of obeticholic acid monotherapy in patients with primary biliary cholangitis. *Hepatology* 2017.
54. Ijssennagger N, Janssen AWF, Milona A, et al. Gene expression profiling in human precision cut liver slices in response to the FXR agonist obeticholic acid. *J Hepatol* 2016; **64**:1158-1166.
55. Alvarez-Sola G, Uriarte I, Latasa MU, et al. Engineered fibroblast growth factor 19 protects from acetaminophen-induced liver injury and stimulates aged liver regeneration in mice. *Cell Death Dis* 2017; **8**:3083.
56. Alvarez-Sola G, Uriarte I, Latasa MU, et al. Fibroblast growth factor 15/19 (FGF15/19) protects from diet-induced hepatic steatosis: development of an FGF19-based chimeric molecule to promote fatty liver regeneration. *Gut* 2017; **66**:1818-1828.
57. Csanaky IL, Aleksunes LM, Tanaka Y, Klaassen CD. Role of hepatic transporters in prevention of bile acid toxicity after partial hepatectomy in mice. *Am J Physiol Gastrointest Liver Physiol* 2009; **297**:419-433.

SUPPLEMENTAL DATA

Figure 1. Activation of hepatic and ileal Fxr-Fgf15 pathways after pre-treatment with OCA, FGF19 or vehicle for seven days



Mice (n=5 mice per group) were pre-treated for 7 days by daily administration of OCA, FGF19 or vehicle, before sacrifice. Transcripts were analysed in the liver **(A, B)**. Values are expressed relative to the median expression in the control group. OCA, obeticholic acid; FGF, fibroblast growth factor; Klb, klotho beta

Chapter 10

General discussion, implications and future perspectives

GENERAL DISCUSSION

Colorectal cancer (CRC) is affecting over 1.3 million patients annually.[1] Approximately 50% of these patients develop colorectal liver metastases (CRLM).[2] In addition, approximately 700 new patients per year in The Netherlands are affected by primary liver cancer (hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA).[3] Partial liver resection for hepatobiliary tumours is often the only curative treatment option.

Despite improved perioperative care, the incidence of postresectional liver failure (PLF) is still 1-9%, due to the increasing complexity and extensiveness of surgical interventions, in combination with an expanding number of resections in patients with compromised liver function. An imbalance between liver volume and quality, with lack of functional recovery after (extended) resection, may lead to PLF. Clinically, liver failure can be characterized by pruritus, jaundice, hepatic encephalopathy, and ascites. Biochemically, elevated serum levels of markers reflecting hepatocellular injury (AST, ALT) and liver transport function (bilirubin) are seen, in combination with a diminished synthetic function (coagulant factors, albumin) of the liver.

Both assessment of liver volume and quality is mandatory to predict postoperative functional reserve. Several risk scores have been developed to detect liver failure postoperatively. Detection of PLF is often too late and treatment is primarily symptomatic.

The central aim of this thesis was to study determinants of, and interventions for postresectional liver (dys)function after partial hepatectomy for liver cancer. This was subdivided into three specific research parts; 1) to define PLF and to study the impact of chemotherapy-associated liver injury on morbidity and mortality after partial hepatectomy for colorectal liver metastases (chapters 2, 3 and 4), 2) to investigate current and future (functional) endpoints to define and detect PLF (chapters 5, 6 and 7), and 3) to examine the role of bile salts and nuclear Farnesoid X Receptor agonism in (the prevention of) liver failure and acceleration of postresectional liver regeneration (chapters 8 and 9).

PART I – PREOPERATIVE DETERMINANTS OF POSTRESECTIONAL LIVER FUNCTION

Liver failure is a feared complication that accounts for up to 75% of mortality after extensive liver resection. Despite improved perioperative care, the increasing complexity and extensiveness of surgical interventions, in combination with an expanding number of resections in patients with compromised liver function, still results in an incidence of PLF

of 1-9%.[4] Preventive measures aim to enhance future remnant liver size and function. Numerous non-invasive techniques to assess liver function and predict remnant liver volume are being developed, along with introduction of novel surgical strategies that augment growth of the future remnant liver.[5] Detection of PLF is often too late and treatment is primarily symptomatic. Current therapeutic research focuses on ([bio] artificial) liver function support and regenerative medicine. In **chapter 2**, we discuss the current state and new developments in prediction, prevention, and management of PLF, in light of novel insights into the aetiology of this complex syndrome.[6]

The first research aim of this thesis was to determine the impact of impaired liver quality due to chemotherapy, on postresectional outcome. Regimens based on the platinum-containing agent oxaliplatin are used extensively as neoadjuvant therapy to downsize initially irresectable colorectal liver metastases, with convincing response rates and survival outcomes.[7-9] However, liver injury reflected in hepatomegaly, ascites and systemic elevation of liver enzymes is demonstrated in over 75% of patients.[10-13] Sinusoidal dilatation is a common manifestation of hepatotoxicity that occurs in patients with colorectal liver metastases (CRLM) after administration of oxaliplatin-based chemotherapy.[14-16] With regard to liver surgery, some studies reported (transient) PLF, higher morbidity rates and impairment of postresectional liver regeneration,[17,18] whereas others could not reproduce this.[19-24] Unfortunately, the majority of articles contained data from relatively small patient groups, varying inclusion criteria and different chemotherapeutical regimens, limiting their validity.

In **chapter 3** we studied the influence of sinusoidal dilatation on short-term outcome after partial hepatectomy for CRLM. We found no significant influence of sinusoidal dilatation on outcome after partial hepatectomy. However, critical evaluation of included evidence by assessment with the QUIPS and GRADE tools, showed a low to high risk of bias for individual studies and very low quality of outcome-specific evidence. This may be explained by suboptimal study design (mostly exploratory phase 1 studies), variation in inclusion criteria, sample size, and wide confidence intervals of the included studies. Moreover, regarding variations in definitions, outcome after liver resection was expressed in multiple terms and time frames. Because of the limited confidence in the provided evidence, no solid conclusions could be drawn, and this study could not provide clinical advice on our topic.

Implications of chapter 3

In 2008, the BMJ published a series of articles on the Grading of Recommendations Assessment, Development and Evaluation (GRADE) system for rating quality of evidence and strength of recommendations that is explicit, comprehensive, transparent, and prag-

matic, and is increasingly being adopted by (healthcare) organisations worldwide.[25] In the current study, evidence was rated as low according to the GRADE guidelines, amongst other reasons due to a suboptimal study design with different inclusion criteria. Ideally, studies should have similar inclusion criteria for comparison. However, due to the heterogeneous group of treated patients and chemotherapy regimens, this may not be feasible.

In **chapter 4** we aimed to explore whether sinusoidal dilatation (SD), steatosis, and steatohepatitis were 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 following the Moose and PRISMA guidelines. For this, a systematic search was performed in Medline (PubMed) and Embase using a search matrix including the following four categories: liver resection, chemotherapy, tumour type, and outcome. The first publication date was fixed on 2004 because the widely used criteria for scoring SD, steatosis, and steatohepatitis were developed in 2004[10] and 2005.[26] After study identification, all corresponding authors of studies that fulfilled the inclusion criteria were contacted by email for collaboration and sharing data of the published cohort. Anonymized data were imported into IBM SPSS Statistics for analysis. The individual participant data from all studies were pooled and modelled simultaneously, and consequently analysed applying one-step binary logistic regression models. As for missing values, multiple imputations were performed, assuming missing at random. Complete case analysis was also conducted for sensitivity analysis.

In this study, increases in postresectional major morbidity (Dindo-Clavien grade ≥3) and liver surgery-specific complications (PLF, intra-abdominal haemorrhage, bile leakage, ascites, intra-abdominal abscess, and mortality) after partial hepatectomy, were observed in patients with SD and steatohepatitis, whereas steatosis was associated with a decreased occurrence of complications. Moreover, PLF occurred more often in patients with severe SD. With respect to steatohepatitis, lobular inflammation, but not severe steatosis or hepatocellular ballooning, was strongly linked to increased postresectional morbidity. Oxaliplatin-based chemotherapy was the sole factor independently associated with an increase in the occurrence of severe SD.

The addition of angiogenesis inhibitor bevacizumab, to oxaliplatin-based chemotherapy has been associated with a decreased incidence of SD.[27,28] In our study we confirmed that bevacizumab was associated with a remarkably decreased occurrence of severe SD. Moreover, an inverse relationship between severe SD and severe steatosis of the liver was found.

Implications of chapter 4

Parenchymal damage due to chemotherapy can be preoperatively diagnosed by radiological and biochemical variables, as reviewed recently in detail by our group.[29] Considering the negative relationship between chemotherapy-associated liver injury (CALI) and postresectional morbidity, it is advised to evaluate parenchymal quality before surgery. Liver elasticity for example can be measured by imaging options such as liver ultrasonographic elastography (FibroScan). Steatohepatitis can be seen on a plain ultrasonography, but also more specific imaging modalities such as Gadolinium MRI have proven to be useful. A biopsy was shown to have little value in the preoperative diagnosis of steatohepatitis due to the heterogeneous spread of histopathological deviations in the liver, and is therefore not recommended.[30]

Moreover, if liver injury is diagnosed preoperatively, it is advised to adapt surgical management when CALI is diagnosed, i.e. central venous pressure should be low during surgery to prevent excessive blood loss. For the same reason the use of radiofrequency ablation might be beneficial when feasible. If there is an indication for evaluation of liver quality during surgery, for example when the liver appears blue (linked to SOS) or yellow (associated with steatosis), frozen sections can be assessed to support the decision to change the type of resection perioperatively. Moreover, with decreased chemotherapy responsiveness,[31,32] shortened overall survival,[18] and increasing doubts about the usefulness of neoadjuvant chemotherapy in certain patient groups,[33] one could even speculate that some patients would benefit from immediate resection instead of neoadjuvant chemotherapeutical treatment. Especially in patient in whom tumour shrinkage is not mandatory, it would be interesting to evaluate the effect of immediate resection on postresectional morbidity and (disease free) survival.

PART II – POSTRESECTIONAL DETECTION OF LIVER DYSFUNCTION

In the era of a declining event-rate, the conduct of a sound trial on liver-surgery specific complications with a dichotomous endpoint (e.g. mortality) would require large sample sizes.[34] The introduction of surrogate endpoints (SEPs) in RCTs is considered a potential solution for solving this problem.[9] A SEP is a laboratory measurement or a physical sign used as a substitute for a clinically meaningful endpoint that measures directly how a patient feels, functions or survives.[10-12] Ideally, changes in a SEP induced by a therapy, should reflect a clinically meaningful endpoint.

In **chapter 5** we aimed to summarize the SEPs representing the effect of surgically induced damage that are being used in liver surgery trials. Additionally, this study aimed at finding common definitions of the employed SEPs, and at recapitulating the evidence or validation justifying the use of those endpoints. Most studies used biologically plausible, though not validated, surrogate outcomes. We were able to retrieve references rationalizing the selection of SEPs used in the majority of the studies. These references were studies using similar endpoints either in clinical trials or in experimental settings. Unfortunately, no study using validated SEPs was found. The selected endpoints all describe alterations either in hepatic or systemic parameters. However, we were not able to verify the relationship between the surrogate and the clinical endpoint or determine parallel estimates of risks and benefits between the surrogate and clinical endpoint. This challenged the validity of the obtained results in the studies included in this review.

In **chapter 6** we validated one of the most used SEPs for PLF. In 2007, Mullen et al. proposed a definition for PLF based on analysis of 1,059 patients without cirrhosis, who underwent major hepatectomy between 1995 and 2005 at three hepatobiliary centres in the United States and Italy.[35] The authors stated that the occurrence of a systemic total bilirubin level of >7.0 mg/dL (≥120 µmol/L, '*peak bilirubin* criterion') within 90 days after major hepatectomy provides a sensitivity of 93.3% for liver-related death and an odds ratio (OR) of 250 (95% confidence interval, 25.0 to >1000) for 90-day liver-related mortality.

We hypothesized that following current practice (increasing incidence of NAFLD, complex vascular procedures, extending indications for resection), the criterion would be met more often, and mortality rates would be higher. Therefore, this study aimed to validate the *peak bilirubin* criterion as a SEP regarding major morbidity and liver-related death within 90 days after partial and major hepatectomy in two European tertiary hepatobiliary referral centres. In the present patient cohort (n=956), sensitivity and specificity of the *peak bilirubin* criterion for 90-day liver-related death after major liver resection were 41.2% and 94.6%, respectively, whereas the positive predictive value only reached 22.6%. In multivariable analysis, the *peak bilirubin* criterion (p<0.001, OR=15.9 [95%CI 5.2-48.7]), co-existing moderate-severe steatosis and moderate-severe fibrosis/cirrhosis (p=0.013), ASA score 3-4 (p=0.047), and age (p=0.044) were independent predictors of liver-related death. Moreover, of the 15 patients with a severely elevated bilirubin level, 10 patients survived, and bilirubin levels normalized. In conclusion, the present study found a rather low positive predictive value and sensitivity of the *peak bilirubin* criterion for liver-related mortality within 90 days after major liver resection. Nevertheless, it was still identified as the most risk-bearing factor for postresectional liver-related mortality within 90 days after partial liver resection in multivariable analysis.

Implications of chapter 5 and 6

Composite (CEP) and surrogate outcomes are, if validated, considered adequate alternatives for replacing the standard short-term dichotomous outcome of mortality and morbidity in many medical fields.[34,36-39] After hepatic surgery, a SEP or CEP can indicate (the risk of) hepatic failure. An increased risk may urge the physician to start with curative or supportive therapy with for instance plasmapheresis or albumin dialysis soon after resection. In contrast, it may prevent over-treatment in groups defined as low risk for hepatic failure. It is therefore of key importance to standardize SEP definitions and validate the SEPs used in liver surgery trials. As all the currently used SEPs are yet to be validated, many definitions can be proposed and adapted to the different effects expected from various interventions. For instance in 2014, following our review a validation study was conducted on the predictive value of serum transaminases after liver resection with or without ischemia reperfusion, which showed that serum transaminases should not be used as a surrogate of postoperative outcome.[40] Prospective studies may focus on novel liver function-related parameters such as bile salts, and/or combined functional and volumetric criteria.[41] In addition, one could stress the liver pharmacologically before liver resection in order to evaluate its functional capacity to react on exogenous and endogenous challenges.

One of the important functions of the liver is the defence against diverse forms of (chemical) challenges and intoxications.[42-44] For example, radicals are scavenged through reaction with the liver-derived tripeptide glutathione (GSH). Amongst other functions, GSH reacts with the analgesic acetaminophen (APAP). At high doses, metabolic processing of APAP gives rise to the reactive compound N-acetyl-p-benzoquinone imine and phenoxyl radicals thereof. Both metabolites can be neutralized by reaction with GSH, which may result in a drop in hepatic GSH levels upon high doses of APAP.[45-48] Animal and in vitro studies showed that systemic ophthalmic acid (OPH) levels increased when hepatocellular GSH and its constituent L-cysteine, were depleted in APAP-induced hepatotoxicity models.[49] OPH lacks a reactive thiol group and is thus devoid of antioxidant properties. It has been suggested that OPH makes use of the same transporter system as GSH and therefore would minimize cellular GSH efflux to preserve cell integrity.[49] Since L-cysteine availability is considered the rate-limiting factor in hepatic GSH formation, elevated plasma OPH concentrations may be a read-out for hepatic GSH depletion.

In **chapter 7** we investigated whether plasma OPH is useful as a read-out for hepatic GSH depletion in humans, by stressing hepatic detoxification capacity with APAP challenges during pylorus-preserving pancreaticoduodenectomy (PPPD) or partial hepatectomy (PH). Nineteen patients undergoing PPPD (n=7, control group) or PH (n=12) were included. APAP (1000 mg) was administered intravenously before resection (first challenge), and six and twelve hours later, with sequential blood sampling during this

period. Arterial, hepatic and portal venous blood samples and liver biopsies were taken on three occasions during the first APAP challenge. Plasma and hepatic OPH and GSH levels were quantified, and venous-arterial differences were calculated to study hepatic release. Our main finding is that systemic GSH levels decreased during APAP challenges in both surgical groups, but this was not accompanied by a reciprocal increase in plasma OPH. Hepatic GSH, OPH and thiyl radicals were not affected within ~3 hours after administration of the first APAP dose in patients undergoing PPPD or PH. Although the liver is considered the predominant source of GSH in the circulation,[43] we did not observe net hepatic GSH release prior to, or after APAP administration in the present study. This indicates that APAP did not result in acute oxidative stress or prompt alterations in hepatic GSH homeostasis. In this period, net release of OPH by the liver was observed only in patients undergoing PPPD.

Implications of chapter 7

The current human model did not prove useful for dynamic prediction of liver function. In the setting of an APAP overdose, serum levels of OPH may be measured to substantiate our proof of concept. If increased OPH serum levels are found, this may function as an early marker of liver injury. It would be interesting to investigate if these serum levels are increased before other liver enzymes such as ALT and AST are increased, and if they correlate with the severity of the injury as confirmed by the clinical condition of the patient, other liver enzymes and imaging features of the liver. If so, the use of serum OPH as a risk indicator early after APAP intoxication should be validated. Once validated, this new marker ought to be part of the risk stratification system for acute liver failure and timing of treatment start (e.g. Molecular Adsorbent Recirculating System (MARS), or plasmapheresis).

PART III – MONITORING AND PREVENTION OF POSTRESECTIONAL LIVER DYSFUNCTION

PLF is a serious complication following partial hepatectomy (PH) with high morbidity and mortality. To find an appropriate therapy to treat and/or prevent the occurrence of liver failure after resection, an animal model would be of great value. We hypothesized that excessive accumulation of bile salts in the regenerating liver remnant, is the actual culprit in PLF.

To study this, we induced bile salt overload in the regenerating liver of mice by feeding them the bile salt cholic acid (CA) after 70% PH (**chapter 8**). Concentrations of CA in the diet ranged from 0.0 to 1.0%, and mice were sacrificed around the time of maximal

hepatocyte proliferation (normally peaking between 36-48 hrs). Mice in the highest dose group had poorer 'clinical' performance (i.e. squinted eyes, reduced physical activity) as indicated by our welfare assessment, with overall decreased glucose levels after PH, and more pronounced body weight loss in the postresectional course. Moreover, in this group, assessment of injury (transaminases) and secretory function (total bilirubin) of the liver, revealed hepatic injury and impaired secretory function. Although no effect of the 1.0 % CA diet was seen on liver mass recovery, impaired hepatocyte proliferation was noted. In conclusion, a postresectional challenge with 1.0% CA diet induces signs of liver injury and defective liver regeneration. A longer duration of the dietary challenge and/or secondary hits may further improve the model. Once validated, it can be used to evaluate pharmaceutical strategies to prevent or treat PLF.

Bile salts have emerged as essential signalling molecules in liver regeneration after PH. Data from animal experiments indicate that endogenous ligands (i.e. bile salts) can stimulate liver regeneration and prevent liver injury after PH, via the hepatic Fxr and ileal Fxr-Fgf15 axis.

In **chapter 9** we investigated whether exogenous activation of the Fxr pathway with the potent semi-synthetic bile acid obeticholic acid (OCA) could stimulate postresectional liver regeneration in mice. In the first part of this study, groups of mice were pre-treated with vehicle or OCA, and sacrificed within 3 days after PH. Unexpectedly, we observed a steep decline in body weight and glycemia after PH in the OCA-treated group. This led us to consider that metabolic effects related to pre-treatment with OCA, masked a potential beneficial effect on postresectional liver regeneration. We therefore replenished drinking water with sucrose for OCA-treated mice in the second part of the study, whilst mice receiving intraperitoneal injections of FGF19 served as a positive control group.[50]

It was anticipated that Fxr activation would result in an earlier peak of regenerative indices through direct (i.e., mitogenic) and indirect (i.e., bile salt homeostatic) effects. We observed inconsistent effects of OCA on regenerative indices in hepatectomized mice. Although responses of Cyp7a1, Cyp8b1 and other Fxr targets implied general effectiveness of OCA treatment, no effect was observed on liver mass recovery after PH. Moreover, OCA had no consistent effect on the number of proliferating (i.e., Ki67+) hepatocytes and mitotic figures at 48 hours after PH. Hepatic bile salt content did not improve around the peak of proliferation at 48 hrs after PH. Besides, welfare of the animals was decreased in OCA-treated animals after PH. After pre-treatment of mice with FGF19, a reduced ileal expression of bile salt-regulated genes Fgf15 and Slc51b indicating FGF19-mediated repression of bile salt synthesis was seen, but this or treatment with FGF19 per se, did not stimulate postresectional liver regeneration in mice.

Implications of chapter 8 and 9

In previous studies it is suggested that bile salts play an important role in liver failure and liver regeneration. Although bile salts seem required for proper postresectional liver regeneration, a tight control of intracellular levels seems indispensable. After liver resection, the remnant liver faces a relative overload of bile salts because the original bile salt pool passes through a smaller liver remnant that apparently has insufficient spare capacity to properly handle this increment. This results in increased systemic spill-over and elevation of circulating bile salts. In our first study, we observed that addition of cholic acid after 70% hepatectomy indeed induced morbidity and liver injury mimicking PLF in an otherwise safe liver resection model.

Next, we hypothesized that optimizing bile salt homeostasis by stimulating its hepatocellular receptor FXR, would accelerate liver regeneration after partial hepatectomy. After administration of the FXR agonist obeticholic acid, we observed little to none beneficial effects on liver regeneration. In our opinion, this indicates that in a normal liver background, liver regeneration after 70% PH already progresses optimally, likely through balanced bile salt homeostasis and effective endogenous bile salt signalling, and cannot be further enhanced.

In clinical practice, however, the indications for liver surgery continue to extend beyond 70% PH in a normal background using vascular procedures, split surgery procedures such as ALPPS permitting resection of larger volumes,[51] and resection performed on livers with impaired quality. These new indications result in more patients being eligible for surgery, however also go along with specific risks during and after surgery. Especially in patients with pre-existent cholestasis due to bile duct obstruction in peri-hilar cholangiocarcinoma, we observe high complication rates.[52] Preoperative attempts to restore bile flow by placing an external drain (PTCD) had detrimental effects on postoperative mortality, indicating that a certain amount of bile salts is indeed required for postoperative liver regeneration.[53,54] In case of surgical removal of a cholangiocarcinoma, the gallbladder is removed perioperatively and a hepaticojejunostomy is created. This may result in depletion of the bile salt pool and inefficient endogenous bile salt signalling in the liver remnant. In this setting, optimization of bile salt homeostasis/signalling, pre-or postoperatively by FXR agonism, may accelerate liver regeneration and proof the indispensable role of bile salt signalling in this process. This concept could be tested in experimental animals, where the endogenous bile salt pool is depleted by a bile salt-binding resin or pharmacological inhibition of intestinal bile salt re-uptake. Under these experimental conditions, we anticipate that FXR agonism can augment liver regeneration after 70% PH.

REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; **136**:359-386.
2. Kanas GP, Taylor A, Primrose JN, et al. Survival after liver resection in metastatic colorectal cancer: review and meta-analysis of prognostic factors. *Clin Epidemiol* 2012; **4**:283-301.
3. IKNL – Cijfers over kanker. Incidentie | Lever en intrahepatische galwegen, 2018.
4. van den Broek MA, Olde Damink SW, Dejong CH, et al. Liver failure after partial hepatic resection: definition, pathophysiology, risk factors and treatment. *Liver Int* 2008; **28**:767-780.
5. Schadde E, Ardiles V, Robles-Campos R, et al. Early survival and safety of ALPPS: first report of the International ALPPS Registry. *Ann Surg* 2014; **260**:829-836.
6. Michalopoulos GK. Liver regeneration. *J Cell Physiol* 2007; **213**:286-300.
7. Lam VW, Spiro C, Laurence JM, et al. A systematic review of clinical response and survival outcomes of downsizing systemic chemotherapy and rescue liver surgery in patients with initially unresectable colorectal liver metastases. *Ann Surg Oncol* 2012; **19**:1292-1301.
8. Seium Y, Stupp R, Ruhstaller T, et al. Oxaliplatin combined with irinotecan and 5-fluorouracil/leucovorin (OCFL) in metastatic colorectal cancer: a phase I-II study. *Ann Oncol* 2005; **16**:762-766.
9. Adam R, Delvart V, Pascal G, et al. Rescue surgery for unresectable colorectal liver metastases downstaged by chemotherapy: a model to predict long-term survival. *Ann Surg* 2004; **240**:644-657.
10. Rubbia-Brandt L, Audard V, Sartoretti P, et al. Severe hepatic sinusoidal obstruction associated with oxaliplatin-based chemotherapy in patients with metastatic colorectal cancer. *Ann Oncol* 2004; **15**:460-466.
11. Robinson SM, Wilson CH, Burt AD, Manas DM, White SA. Chemotherapy-associated liver injury in patients with colorectal liver metastases: a systematic review and meta-analysis. *Ann Surg Oncol* 2012; **19**:4287-4299.
12. Overman MJ, Maru DM, Charnsangavej C, et al. Oxaliplatin-mediated increase in spleen size as a biomarker for the development of hepatic sinusoidal injury. *J Clin Oncol* 2010; **28**:2549-2555.
13. Arotcarena R, Cales V, Berthelemy P, et al. Severe sinusoidal lesions: a serious and overlooked complication of oxaliplatin-containing chemotherapy? *Gastroenterol Clin Biol* 2006; **30**:1313-1316.
14. Cancer IAfRo. GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012. Cancer fact sheets, colorectal cancer.
15. Landelijke werkgroep Gastro Intestinale Tumoren I. Colorectaalcarcinoom, Versie: 3.0. 2014: 1-226
16. Nordlinger B, Van Cutsem E, Rougier P, et al. Does chemotherapy prior to liver resection increase the potential for cure in patients with metastatic colorectal cancer? A report from the European Colorectal Metastases Treatment Group. *Eur J Cancer* 2007; **43**:2037-2045.
17. Nakano H, Oussoultzoglou E, Rosso E, et al. Sinusoidal injury increases morbidity after major hepatectomy in patients with colorectal liver metastases receiving preoperative chemotherapy. *Ann Surg* 2008; **247**:118-124.
18. Tamandl D, Klinger M, Eipeldauer S, et al. Sinusoidal obstruction syndrome impairs long-term outcome of colorectal liver metastases treated with resection after neoadjuvant chemotherapy. *Ann Surg Oncol* 2011; **18**:421-430.
19. Kandutsch S, Klinger M, Hacker S, Wrba F, Gruenberger B, Gruenberger T. Patterns of hepatotoxicity after chemotherapy for colorectal cancer liver metastases. *Eur J Surg Oncol* 2008; **34**:1231-1236.
20. Komori H, Beppu T, Baba Y, et al. Histological liver injury and surgical outcome after FOLFOX followed by a hepatectomy for colorectal liver metastases in Japanese patients. *Int J Clin Oncol* 2010; **15**:263-270.

21. Makowiec F, Mohrle S, Neeff H, et al. Chemotherapy, liver injury, and postoperative complications in colorectal liver metastases. *J Gastrointest Surg* 2011; **15**:153-164.
22. Pawlik TM, Olino K, Gleisner AL, Torbenson M, Schulick R, Choti MA. Preoperative chemotherapy for colorectal liver metastases: impact on hepatic histology and postoperative outcome. *J Gastrointest Surg* 2007; **11**:860-868.
23. Sahajpal A, Vollmer CM, Dixon E, et al. Chemotherapy for colorectal cancer prior to liver resection for colorectal cancer hepatic metastases does not adversely affect peri-operative outcomes. *J Surg Oncol* 2007; **95**:22-27.
24. Takeda D, Nitta H, Takahara T, Hasegawa Y, Itou N, Wakabayashi G. Effect of preoperative chemotherapy on postoperative liver regeneration following hepatic resection as estimated by liver volume. *World J Surg Oncol* 2013; **11**:65.
25. Guyatt GH, Oxman AD, Vist GE, et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. *BMJ* 2008; **336**:924-926.
26. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41**:1313-1321.
27. Rubbia-Brandt L, Lauwers GY, Wang H, et al. Sinusoidal obstruction syndrome and nodular regenerative hyperplasia are frequent oxaliplatin-associated liver lesions and partially prevented by bevacizumab in patients with hepatic colorectal metastasis. *Histopathology* 2010; **56**:430-439.
28. Ribero D, Wang H, Donadon M, et al. Bevacizumab improves pathologic response and protects against hepatic injury in patients treated with oxaliplatin-based chemotherapy for colorectal liver metastases. *Cancer* 2007; **110**:2761-2767.
29. van Mierlo KM, Schaap FG, Dejong CH, Olde Damink SW. Liver resection for cancer: New developments in prediction, prevention and management of postresectional liver failure. *J Hepatol* 2016; **65**:1217-1231.
30. Vigano L, Ravarino N, Ferrero A, Motta M, Torchio B, Capussotti L. Prospective evaluation of accuracy of liver biopsy findings in the identification of chemotherapy-associated liver injuries. *Arch Surg* 2012; **147**:1085-1091.
31. Vreuls CP, Van Den Broek MA, Winstanley A, et al. Hepatic sinusoidal obstruction syndrome (SOS) reduces the effect of oxaliplatin in colorectal liver metastases. *Histopathology* 2012; **61**:314-318.
32. Vigano L, Capussotti L, De Rosa G, De Saussure WO, Mentha G, Rubbia-Brandt L. Liver Resection for Colorectal Metastases after Chemotherapy Impact of Chemotherapy-Related Liver Injuries, Pathological Tumor Response, and Micrometastases on Long-term Survival. *Ann Surg* 2013; **258**:731-742.
33. Khoo E, O'Neill S, Brown E, Wigmore SJ, Harrison EM. Systematic review of systemic adjuvant, neoadjuvant and perioperative chemotherapy for resectable colorectal-liver metastases. *HPB* 2016; **18**:485-493.
34. van den Broek MA, van Dam RM, van Breukelen GJ, et al. Development of a composite endpoint for randomized controlled trials in liver surgery. *Br J Surg* 2011; **98**:1138-1145.
35. Mullen JT, Ribero D, Reddy SK, et al. Hepatic insufficiency and mortality in 1,059 noncirrhotic patients undergoing major hepatectomy. *J Am Coll Surg* 2007; **204**:854-862.
36. Ellenberg S, Hamilton JM. Surrogate endpoints in clinical trials: cancer. *Stat Med* 1989; **8**:405-413.
37. Fleming TR. Surrogate markers in AIDS and cancer trials. *Stat Med* 1994; **13**:1423-1435.
38. Freemantle N, Calvert M. Composite and surrogate outcomes in randomised controlled trials. *BMJ* 2007; **334**:756-757.
39. Espeland MA, O'Leary D H, Terry JG, Morgan T, Evans G, Mudra H. Carotid intimal-media thickness as a surrogate for cardiovascular disease events in trials of HMG-CoA reductase inhibitors. *Curr Control Trials Cardiovasc Med* 2005; **6**:3.

40. Boleslawski E, Vibert E, Pruvot FR, et al. Relevance of postoperative peak transaminase after elective hepatectomy. *Ann Surg* 2014; **260**:815-820.
41. DuBray BJ, Jr., Levy RV, Balachandran P, et al. Novel three-dimensional imaging technique improves the accuracy of hepatic volumetric assessment. *HPB* 2011; **13**:670-674.
42. Hinson JA, Reid AB, McCullough SS, James LP. Acetaminophen-induced hepatotoxicity: role of metabolic activation, reactive oxygen/nitrogen species, and mitochondrial permeability transition. *Drug Metab Rev* 2004; **36**:805-822.
43. Biolo G, Antonione R, De Cicco M. Glutathione metabolism in sepsis. *Crit Care Med* 2007; **35**(9 Suppl):591-595.
44. Hammond CL, Lee TK, Ballatori N. Novel roles for glutathione in gene expression, cell death, and membrane transport of organic solutes. *J Hepatol* 2001; **34**:946-954.
45. Jaeschke H, McGill MR, Ramachandran A. Oxidant stress, mitochondria, and cell death mechanisms in drug-induced liver injury: lessons learned from acetaminophen hepatotoxicity. *Drug Metab Rev* 2012; **44**:88-106.
46. McGill MR, Lebofsky M, Norris HR, et al. Plasma and liver acetaminophen-protein adduct levels in mice after acetaminophen treatment: dose-response, mechanisms, and clinical implications. *Toxicol Appl Pharmacol* 2013; **269**:240-249.
47. Ramakrishna Rao DN, Fischer V, Mason RP. Glutathione and ascorbate reduction of the acetaminophen radical formed by peroxidase. Detection of the glutathione disulfide radical anion and the ascorbyl radical. *J Biol Chem* 1990; **265**:844-847.
48. Jaeschke H, Williams CD, Ramachandran A, Bajt ML. Acetaminophen hepatotoxicity and repair: the role of sterile inflammation and innate immunity. *Liver Int* 2012; **32**:8-20.
49. Soga T, Baran R, Suematsu M, et al. Differential metabolomics reveals ophthalmic acid as an oxidative stress biomarker indicating hepatic glutathione consumption. *J Biol Chem* 2006; **281**:16768-16776.
50. Uriarte I, Fernandez-Barrena MG, Monte MJ, et al. Identification of fibroblast growth factor 15 as a novel mediator of liver regeneration and its application in the prevention of post-resection liver failure in mice. *Gut* 2013; **62**:899-910.
51. Schadde E, Malago M, Hernandez-Alejandro R, et al. Monosegment ALPPS hepatectomy: extending resectability by rapid hypertrophy. *Surgery* 2015; **157**:676-689.
52. van Golen RF, Olthof PB, Lionarons DA, et al. FXR agonist obeticholic acid induces liver growth but exacerbates biliary injury in rats with obstructive cholestasis. *Sci Rep* 2018; **8**:16529.
53. Coelen RJS, Roos E, Wiggers JK, et al. Endoscopic versus percutaneous biliary drainage in patients with resectable perihilar cholangiocarcinoma: a multicentre, randomised controlled trial. *Lancet Gastroenterol Hepatol* 2018; **3**:681-690.
54. Otao R, Beppu T, Isiko T, et al. External biliary drainage and liver regeneration after major hepatectomy. *BJS* 2012; **99**:1569-1574.

Appendix

SUMMARY

Chapter 1 is a general introduction describing the aims of this thesis. Postresectional liver failure (PLF) is a feared complication after liver resection for primary liver cancer or colorectal cancer liver metastases. It comprises of impaired secretory, detoxifying, and synthetic function of the liver. Multiple definitions of liver failure are used in studies on outcomes after partial liver resection. We assessed the current definitions and functional endpoints of PLF. The aetiology of this complex syndrome lies, amongst others, in a disbalance of remaining quality and quantity of liver tissue, and disturbed bile salt homeostasis. We therefore investigated the role of impaired liver quality due to chemotherapy-induced liver injury on the prevalence of PLF. Next, we created a mouse model to study liver failure, and examined the role of bile salts and bile salt receptor (FXR) agonism in (the prevention of) liver failure and acceleration of postresectional liver regeneration.

In chapter 2, we discuss the current state and new developments in prediction, prevention, and management of PLF, in light of novel insights into the aetiology of this complex syndrome. Despite improved perioperative care, the increasing complexity and extensiveness of surgical interventions, in combination with an expanding number of resections in patients with compromised liver function, still results in an incidence of PLF of 1–9%. Preventive measures aim to enhance future remnant liver size and function. Numerous non-invasive techniques to assess liver function and predict remnant liver volume are being developed, along with introduction of novel surgical strategies that augment growth of the future remnant liver. Detection of PLF is often too late and treatment is primarily symptomatic. Current therapeutic research focuses on ([bio] artificial) liver function support and regenerative medicine.

Chemotherapy is often used as neoadjuvant therapy to downsize initially irresectable colorectal liver metastases. However, liver injury is demonstrated in most patients. Sinusoidal dilatation occurs in patients with colorectal liver metastases after administration of oxaliplatin-based chemotherapy. In **chapter 3** we studied the influence of sinusoidal dilatation (SD) on short-term outcome after partial hepatectomy for CRLM. Multiple online databases were searched for studies published between 01.01.2004 and 09.06.2015. We included studies comprising adults who underwent partial hepatectomy for CRLM with grading of SD and registration of postoperative morbidity and/or mortality. Meta-analysis on the available data showed that there was no significant influence of SD on overall morbidity, PLF, overall mortality, and liver-related morbidity after partial hepatectomy. However, critical evaluation of included evidence by assessment with the QUIPS and GRADE tools, showed a very low quality of outcome-specific evidence. This may be explained by a.o. suboptimal study design and variation in inclusion criteria and outcomes.

Because of the limited confidence in the provided evidence, this study could not provide clinical advice on our topic.

In **chapter 4** we aimed to explore whether SD), steatosis, and steatohepatitis were associated with increased morbidity and mortality rates after partial hepatectomy. To increase confidence in our evidence, we performed a systematic literature review following the Moose and PRISMA guidelines and contacted all authors of the included articles. A large dataset of multiple international centers with individual participant data was created, and we performed uni- and multivariable analyses. We observed increased postresectional major morbidity and liver surgery-specific complications after partial hepatectomy in patients with SD and steatohepatitis, whereas steatosis was associated with a decreased occurrence of complications. Moreover, PLF occurred more often in patients with severe SD. With respect to steatohepatitis, lobular inflammation was strongly linked to increased postresectional morbidity. 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.

In the era of a declining event-rate, a sound trial on liver-surgery specific complications with a dichotomous endpoint requires large sample sizes. Surrogate endpoints (SEPs) are considered a potential solution for this problem. A SEP is a laboratory measurement or a physical sign used as a substitute for a clinically meaningful endpoint that measures directly how a patient feels, functions or survives. In **chapter 5** we aimed to summarize and validate the SEPs of a total of 49 articles representing the effect of surgically induced damage that are being used in liver surgery trials. Standard biochemical liver functions tests were the most frequently used SEPs. The used definitions of SEPs varied greatly among the studies. Although we found rationalizing the selection of SEPs used in most of the studies, no validating studies were found. Therefore, we were not able to verify the relationship between the surrogate and the clinical endpoint or determine parallel estimates of risks and benefits between the surrogate and clinical endpoint.

In 2007, Mullen stated that the occurrence of a systemic total bilirubin level of >7.0 mg/dL (≥120 µmol/L, '*peak bilirubin* criterion') within 90 days after major hepatectomy provides a positive predictive value of 32.6% for liver-related death. In **chapter 6** we validated the peak bilirubin criterion as one of the most used SEPs for postresectional liver-related mortality. Patient and surgical characteristics of 956 consecutive patients who underwent partial hepatectomy in two European centers were analysed by uni- and multivariable analyses. Sensitivity and specificity for liver-related mortality after major hepatectomy were 41.2% and 94.6%, respectively. Although the peak bilirubin criterion

was an independent predictor, the positive predictive value was only 22.6% for 90-day liver-related mortality after major hepatectomy.

One of the important functions of the liver is the defence against (chemical) challenges and intoxications. Radicals are scavenged through reaction with the liver-derived tripeptide glutathione (GSH), an important determinant of liver function. Animal studies indicate that systemic ophthalmic acid (OPH) is a biomarker for hepatic glutathione (GSH) homeostasis. In **chapter 7** we investigated whether plasma OPH is useful as a read-out for hepatic GSH depletion in humans. We stressed hepatic detoxification capacity with APAP challenges during pylorus-preserving pancreaticoduodenectomy (PPPD) or partial hepatectomy (PH) in nineteen patients. Our main finding is that systemic GSH levels decreased during APAP challenges in both surgical groups, but this was not accompanied by a reciprocal increase in plasma OPH. Hepatic GSH, OPH and thiyl radicals were not affected after administration of the first APAP dose in patients undergoing PPPD or PH. We did not observe net hepatic GSH release prior to, or after APAP administration in the present study. This indicates that APAP did not result in acute oxidative stress or prompt alterations in hepatic GSH homeostasis, and it seems thus unlikely that hepatic GSH homeostasis was sufficiently challenged in the present study.

To find an appropriate therapy to treat and/or prevent the occurrence of liver failure after resection, an experimental animal model would be of great value. Postresectional hyperbilirubinemia suggests that impaired hepatobiliary transport with intrahepatic accumulation of harmful cholephiles plays an etiological role. We hypothesized that excessive accumulation of bile salts in the regenerating liver remnant is the actual culprit in PLF. To study this, we induced bile salt overload in the regenerating liver of twelve weeks old male C57BL6/J mice by feeding them a diet supplemented with cholic acid (CA, 0.5 or 1.0%) or control diet after 70% PH (**chapter 8**). Mice were sacrificed at 48 hours, thus around the time of maximal hepatocyte proliferation. Mice fed a 1.0% CA diet displayed more pronounced weight loss, had poorer 'clinical' performance and overall decreased glucose levels after PH. Moreover, liver injury and impaired hepatobiliary transport function were apparent in the group fed a 1.0% CA diet, but not in animals fed a 0.5% CA diet. Although no effect of the 1.0 % CA diet was seen on liver mass recovery, impaired hepatocyte proliferation was noted.

Although a bile salt overload seems to induce PLF, data from animal experiments indicate that bile salt signaling via the Fxr-Fgf15 axis is required for liver regeneration and prevention of liver injury after PH. In **chapter 9** we investigated whether exogenous activation of the Fxr pathway with the potent semi-synthetic bile acid obeticholic acid (OCA) could stimulate postresectional liver regeneration in mice. In the first part of this study, groups

of twelve weeks old mice were pre-treated with OCA or vehicle and sacrificed after 70% PH. Unexpectedly, we observed a steep decline in body weight and glycemia after PH in the OCA-treated group. We therefore replenished drinking water with sucrose for OCA-treated mice in the second part of the study. We also included a group of mice receiving intraperitoneal injections of FGF19 as a positive control group. No effect could be detected on liver mass recovery or number of proliferating hepatocytes after PH, although responses of Cyp7a1, Cyp8b1 and other Fxr target genes implied general effectiveness of OCA treatment. PH. Serum and liver bile salt content were not influenced by treatment before and after PH. Besides, welfare was decreased in OCA-treated animals after PH. After pre-treatment of mice with FGF19, FGF19-mediated repression of bile salt synthesis was seen, but this did not stimulate postresectional liver regeneration in mice. In our opinion, this indicates that in a normal liver background, liver regeneration after 70% PH already progresses optimally, likely through balanced bile salt homeostasis and effective endogenous bile salt signalling.

SAMENVATTING

Hoofdstuk 1 is een algemene inleiding waarin de doelstellingen van dit proefschrift worden beschreven. Postresectioneel leverfalen (PLF) is een gevreesde complicatie na partiële hepatectomie (PH) voor primaire leverkanker of levermetastasen bij colorectale kanker. Het bestaat uit een verstoorde secretoire, detoxificerende en synthetische functie van de lever. Meerdere definities van leverfalen worden gebruikt in onderzoeken naar uitkomsten van leverresecties. We hebben de huidige definities en functionele eindpunten van PLF beoordeeld. De etiologie van dit complexe syndroom ligt onder meer in een disbalans van de resterende kwantiteit en kwaliteit van het leverweefsel en een verstoorde homeostase van galzouten. We onderzochten daarom de rol van de verminderde leverkwaliteit als gevolg van chemotherapie-geïnduceerde leverschade op de prevalentie van PLF. Vervolgens creëerden we een muismodel om leverfalen te bestuderen, en onderzochten we de rol van galzouten en nucleair Farnesoid X Receptor-agonisme bij (de preventie van) leverfalen en versnelling van postresectionele leverregeneratie.

In **hoofdstuk 2** onderzoeken we de huidige stand van zaken en ontwikkelingen in de voorspelling, preventie en behandeling van PLF. Ondanks de verbetering in perioperatieve zorg, zorgen de toenemende complexiteit en uitgebreidheid van chirurgische interventies in combinatie met het toenemende aantal resecties in patiënten met een verminderde leverfunctie, voor een incidentie van PLF van 1-9%. Interventies zijn erop gericht het toekomstige levervolume te vergroten en de functie te optimaliseren. Meerdere niet-invasieve technieken die de leverfunctie in kaart brengen en het toekomstige levervolume voorspellen zijn in ontwikkeling, samen met nieuwe chirurgische strategieën die de groei van de toekomstige lever stimuleren. Detectie van PLF is vaak laat en de behandeling is primair symptomatisch. Huidige behandelstrategieën richten zich op ondersteuning van de leverfunctie en de regeneratieve geneeskunde.

Chemotherapeutica worden vaak neo-adjuvant gebruikt om initieel inoperatieve colorectale levermetastasen te verkleinen. Dit kan echter ook leverschade tot gevolg hebben. Dilatatie van de sinusoïden komt vaker voor bij patiënten met colorectale levermetastasen na het gebruik van oxaliplatine. In **hoofdstuk 3** bestudeerden we de invloed van sinusoïdale dilatatie (SD) op korte-termijn uitkomsten na PH voor colorectale levermetastasen. In meerdere online databases zochten we naar studies in volwassenen die tussen 2004 en 2015 PH ondergingen voor colorectale levermetastasen en waarbij de korte termijnuitkomsten en leverschade waren gedocumenteerd. Meta-analyses op de beschikbara data lieten zien dat er geen significante invloed was van sinusoïdale dilatatie op morbiditeit, leverfalen, mortaliteit en lever-gerelateerde morbiditeit na PH. Evaluatie van de geïncludeerde literatuur middels de QUIPS en GRADE scorelijsten lieten echter een lage

kwaliteit van het bewijs zien. Dit kon onder andere verklaard worden door de suboptimale opzet van studies en variatie in inclusiecriteria. Door de verminderde betrouwbaarheid van ons bewijs, konden we geen conclusies trekken.

In **hoofdstuk 4** onderzochten we of SD, steatose en steatohepatitis geassocieerd waren met verhoogde morbiditeit en mortaliteit na PH. Om het vertrouwen in het bewijsmateriaal te vergroten, voerden we een systematisch literatuuronderzoek volgens de Moose- en PRISMA-richtlijnen uit en namen contact op met de auteurs van de geïncludeerde artikelen. We creëerden een grote dataset van meerdere internationale centra met individuele deelnemersgegevens waarop we uni- en multivariabele analyses uitvoerden. We observeerden verhoogde postoperatieve ernstige morbiditeit en leveroperatie-specifieke complicaties na PH bij patiënten met SD en steatohepatitis, terwijl steatose geassocieerd was met een verminderd optreden van complicaties. Bovendien kwam PLF vaker voor bij patiënten met ernstige SD. Oxaliplatine was sterk gerelateerd aan een verhoogd optreden van ernstige SD, en de toevoeging van bevacizumab aan een op oxaliplatine-gebaseerd chemotherapie-regime verminderde het optreden van ernstige SD.

In het tijdperk van een dalend aantal postoperatieve complicaties, vereist een degelijk onderzoek naar leveroperatie-specifieke complicaties met een dichotoom eindpunt een grote omvang van de studiepopulatie. Surrogaateindpunten (SEPs) worden beschouwd als een mogelijke oplossing voor dit probleem. Een SEP is een laboratoriummeting of een fysiek kenmerk van de patient dat wordt gebruikt als vervanging voor een klinisch eindpunt dat direct meet hoe een patiënt zich voelt of functioneert. In **hoofdstuk 5** hebben we getracht SEPs in 49 artikelen over leverchirurgie-geïnduceerde schade te analyseren. Biochemische leverfunctietesten waren de meest gebruikte SEPs. De gebruikte definities van SEPs varieerden sterk tussen de onderzoeken. We vonden geen validerende onderzoeken naar de SEPs, waardoor we niet in staat waren om de relatie tussen het surrogaat en het klinische eindpunt te verifiëren.

In 2007 verklaarde Mullen et al. dat het optreden van een serum bilirubinespiegel van >7,0 mg/dL (≥120 µmol/L, 'piek bilirubine criterium') binnen 90 dagen na uitgebreide hepatectomie een gevoeligheid van 93,3% voor levergerelateerd overlijden geeft, en een positief voorspellende waarde van 33,6%. In **hoofdstuk 6** hebben we het piek bilirubine criterium gevalideerd als een van de meest gebruikte SEPs voor postresectionele levergerelateerde mortaliteit. Patiënt- en chirurgische kenmerken van 956 patiënten die PH ondergingen in twee Europese centra werden geanalyseerd door middel van uni- en multivariabele analyses. De sensitiviteit en specificiteit voor levergerelateerde mortaliteit na uitgebreide hepatectomie waren respectievelijk 41,2% en 94,6%. Hoewel het piek bilirubine criterium

een onafhankelijke voorspeller was, was de positief voorspellende waarde slechts 22,6% voor levergerelateerde mortaliteit binnen 90 dagen na uitgebreide hepatectomie.

Een van de belangrijke functies van de lever is de verdediging tegen (chemische) intoxicaties. Radicalen worden weggevangen door reactie met het tripeptide glutathion (GSH), een belangrijke determinant voor leverfunctie. Dierstudies geven aan dat plasma oftalmaat (OPH) een biomarker is voor glutathion (GSH) homeostase in de lever. In **hoofdstuk 7** hebben we onderzocht of plasma-OPH bruikbaar is als read-out voor lever-GSH-depletie bij mensen. We belastten de detoxificatie capaciteit van de lever met APAP-giften tijdens pylorusbehoudende pancreaticoduodenectomie (PPPD) of PH bij negentien patiënten. Onze belangrijkste bevinding is dat plasma GSH-spiegels tijdens de APAP-giften in beide chirurgische groepen afnamen, maar dit ging niet gepaard met een toename van plasma OPH. Hepatische GSH-, OPH- en thiylradicalen werden niet beïnvloed na toediening van de eerste APAP-dosis bij patiënten die PPPD of PH ondergingen. We namen geen netto hepatische GSH-afgifte waar vóór of na APAP-toediening. Dit geeft aan dat APAP giften niet resulteerden in acute oxidatieve stress of veranderingen in GSH-homeostase in de lever. Het lijkt dus onwaarschijnlijk dat de hepatische GSH-homeostase voldoende werd belast in de huidige studie.

Om een geschikte therapie of preventie te vinden voor postoperatief leverfalen, zou een diermodel van grote waarde zijn. Postoperatieve hyperbilirubinemie suggereert dat een verstoord hepatobiliair transport met intrahepatische accumulatie van schadelijke galzouten een rol speelt. We veronderstelden dat overmatige ophoping van galzouten in het regenererende leverresidu bijdraagt aan de ontwikkeling van PLF. Om dit te bestuderen, induceerden we galzoutoverbelasting in de regenererende lever van mannelijke C57BL6/J-muizen door ze een dieet te geven aangevuld met cholaat (CA, 0,5 of 1,0%) of een controle dieet na 70% hepatectomie (**hoofdstuk 8**). Muizen werden opgeofferd na 48 uur. Muizen die een CA-dieet van 1,0% kregen, vertoonden meer uitgesproken gewichtsverlies, functioneerden klinisch slechter en hadden verlaagde glucosespiegels na PH. Bovendien toonden muizen met een 1,0% CA-dieet leverbeschadiging en verminderde lever- en galtransportfunctie, terwijl dit niet werd gezien in muizen die een 0,5% CA-dieet kregen. Hoewel er geen effect van het 1,0% CA-dieet werd waargenomen op het herstel van de levermassa, werd wel een verminderde proliferatie van hepatocyten geobjectiveerd.

Hoewel een overdosis van galzouten postoperatief leverfalen lijkt te induceren, tonen gegevens uit dierexperimenten dat galzouten wel noodzakelijk zijn voor leverregeneratie en het voorkomen van leverbeschadiging na PH, via de hepatische Fxr- en ileale Fxr-Fgf15-as. In **hoofdstuk 9** hebben we onderzocht of exogene activering van de Fxr-route met het krachtige semi-synthetische obeticholzuur (OCA) postresectionele leverregeneratie bij muizen

kan stimuleren. In het eerste deel van deze studie werden groepen van twaalf weken oude muizen voorbehandeld met OCA of vehiculum en opgeofferd nadat ze 70% PH ondergingen. Onverwachts zagen we in de met OCA behandelde groep een vermindering van het lichaamsgewicht en serum glucose na PH. In het tweede deel van het onderzoek vulden we drinkwater van met OCA behandelde muizen daarom aan met sucrose. Tevens werd als positieve controle een groep muizen opgenomen die intraperitoneale injecties van FGF19 ontving. Hoewel de respons van Cyp7a1, Cyp8b1 en andere Fxr-gerelateerde genen de algemene effectiviteit van OCA-behandeling impliceerden, kon er geen effect worden gevonden op het herstel van de levermassa of het percentage prolifererende hepatocyten na PH. Het galzoutgehalte in serum en lever werden niet beïnvloed door de behandeling voor en na PH. Na voorbehandeling van muizen met FGF19 werd door FGF19 gemedieerde repressie van galzoutsynthese gezien, maar dit stimuleerde postoperatieve leverregeneratie bij muizen niet. Naar onze mening betekent dit dat in een normale leverachtergrond de leverregeneratie na 70% PH al optimaal plaatsvindt, waarschijnlijk door een effectieve en evenwichtige endogene galzoutsignalering.

SCIENTIFIC AND SOCIETAL IMPACT

Colorectal carcinoma is one of the most common type of cancer worldwide, with approximately 1,3 million new patients annually. Almost half of these patients develop metastases in the liver. Also, annually approximately 700 patients develop primary liver cancer in The Netherlands. Surgical removal (partial hepatectomy) of the tumor offers the best chance of cure.

Failure of liver functions is an important complication and develops in around 9% of patients after partial hepatectomy. A shifted balance between the amount of liver tissue that is removed, and the quality of the remaining liver tissue seems to be the causal factor. Treatment of liver failure solely consists of supporting measures, which causes a mortality of 30% in patients developing liver failure. In this thesis, several determinants of liver failure are investigated. We studied 1) the impact of chemotherapy on complication rates after partial hepatectomy, 2) criteria that can be used in practice as a definition and predictor of postoperative liver failure, and 3) the role of bile salt homeostasis in liver failure and postoperative liver regeneration.

The influence of chemotherapy on outcome after partial hepatectomy

Preoperative chemotherapy is administered to reduces the size of liver tumors, making more patients eligible for a (smaller) resection. However, previous studies showed that chemotherapy also affects healthy liver tissue, which results in an enlarged liver, ascites, and elevation of serum liver enzymes. Microscopically, dilatation of the blood vessels in the liver is seen (sinusoidal dilatation). In chapter 4 we investigated the influence of liver injury on the outcomes after liver resection. Unfortunately, the results from the studies published up to that point could not be compared properly due to different inclusion criteria and outcome measures. We therefore advocate the formulation of uniform inclusion criteria for prospective studies, as well as uniform definitions for outcome measures such as liver failure and biliary leakage. This would provide a fairer and more reliable picture of the results. Preferably, outcome values specifically related to quality of life of the patient are also included in this set of outcomes. Initiatives such as the DHBA (Dutch Hepato Biliary Audit) are valuable for the prospective collection of data and the application of retrospective studies on these datasets because all relevant data for comparison of (treatments of) patients can be collected.

To formulate an answer to the abovementioned question, all researchers from previous studies on chemotherapy-associated liver injury were approached to share their data for analysis. Multiple researchers gave permission, resulting in a large database with data on chemotherapy regimens, surgical procedures, and patient characteristics. After analyses,

we were able to conclude that sinusoidal dilatation and steatohepatitis increase the risk of serious general complications and liver-specific complications (such as liver failure, hemorrhage, and bile leakage) after partial hepatectomy. In contrast, fatty liver (steatosis) is associated with fewer complications after partial hepatectomy. Chemotherapy regimens consisting of solely oxaliplatin were associated with sinusoidal dilatation, whilst the addition of bevacizumab appeared to limit this injury. Given the negative impact of chemotherapy-induced injury on the outcome after liver resection, we recommend evaluating the quality of liver tissue before surgery, for example by ultrasound or MRI. If there is evidence of liver injury, the surgical plan may have to be adjusted or postponed, depending on the expected complications. One can also opt for removal of the tumorous part of the liver without pre-operative chemotherapy, or to adjust the type of chemotherapy regimen. Of course, this should be discussed in a multidisciplinary team meeting (MDO), in which the valued opinions of, among others, surgeon, oncologist and radiotherapist should be considered.

Definitions and predictive values of the present criteria for liver failure

Because liver failure after liver resection is rare but can lead to major consequences if it does occur, it is virtually impossible to set up studies with mortality as an end point after liver resection. That is why we investigated the existing 'surrogate endpoints' (SEP): a laboratory value or physical sign that can act as a predictor of a specific complication, so that for example supportive therapy can be initiated earlier in the postoperative course. In chapter 5 we examined the current existing SEPs for their validity as predictors of liver complication-specific endpoints. Unfortunately, we could not verify the relationship between the surrogate endpoint and the clinical endpoint, which means they cannot be used as a predictor in practice. Subsequently, in chapter 6 a widely used SEP, the peak bilirubin (serum bilirubin >120 µmol/L within 90 days after major liver resection) was investigated as a predictor of liver failure and liver-related mortality. In our patient database, peak bilirubin was found to have a predictive value of only 22.6% on liver-related death after major liver surgery. This means that more than 3 in 4 patients will not die after meeting this criterion, and that a high bilirubin value is not a reason for discontinuation of supportive therapy or an infaust prognosis.

One way to test liver quality before resection is to measure its functional capacity with an endogenous or exogenous test. An important substance involved in metabolizing exogenous components in the body is glutathione (GSH). Amongst other things, GSH is involved in the processing of paracetamol (APAP) and its toxic metabolite. Presumably, after administration of (a high dose of) paracetamol, the amount of GSH in the liver decreases, whilst the amount of ophthalmic acid (OPH) in the blood rises. In chapter 7 we investigated whether plasma ophthalmic acid could be a useful read-out for hepatic

GSH depletion by testing the liver's detoxifying capacity during partial hepatectomy. In this study, patients were repeatedly administered a (safe) dose of paracetamol while part of the liver or pancreas was surgically removed. In both groups, no reduction in the amount of GSH in the liver was seen, nor was there a marked increase in plasma OPH. Unfortunately, no prediction of liver function could be made based on these results. We speculate that, in case of paracetamol overdose, plasma OPH levels may indicate liver injury. It would be relevant to investigate whether these levels are elevated earlier than standard measures such as ALT and AST, and whether they correlate with patient outcome (liver failure, death). In this case, assisted therapy such as hemodialysis could be started earlier in the post-intoxication course to improve outcome.

The role of bile salt metabolism in liver failure and liver regeneration

Previous studies suggest that an imbalance of bile salts has a negative influence on liver regeneration after liver resection. Both a deficiency and a (significant) overload impede liver regeneration and induce liver failure, while a pinch of extra bile salts seems to have an accelerating effect on liver regeneration. In chapter 8 we designed a mouse model in which we investigated the role of bile salts in liver failure. Mice were offered different diets with an increasing amount of bile salts. We witnessed the mice with the highest concentration of bile salts in the diet appearing less well clinically and showing signs of liver failure. We intend to use these diets in the future as a model for liver failure, whereby interventions to prevent liver failure can be tested.

In chapter 9, a potential therapy to prevent liver failure was tested. Obeticholic acid (OCA), a semi-synthetic bile salt (approved as a second-line treatment for a specific liver disease), was administered to mice after which they underwent partial hepatectomy. By administering OCA to mice in low doses, we hypothesized that liver regeneration was accelerated after partial hepatectomy. Unfortunately, no consistent results were obtained on accelerated growth of liver mass after surgery, while gene expression analysis showed that important genes for liver regeneration were activated. We did not find a clear effect on the amount of bile salts in the liver. In our opinion, this indicates that the regeneration mechanism in healthy mice is already optimal after resection of 70% of the liver tissue. However, in daily clinical practice, we regularly deal with people who do not have a healthy liver or a 'straightforward' surgical procedure. Draining bile salts from the body before surgery has been proven to have a negative effect on outcome after liver resection. We suspect that in this situation the administration of, for example, a semi-synthetic bile salt such as OCA accelerates and optimizes liver regeneration. In clinic, this could lead to safer liver resections in patients with a percutaneous drain. The ultimate goal of all interventions is to enable safe surgical procedures in patients with an affected liver, making more patients eligible for a cure, with fewer complications.

DANKWOORD

Hora est! Het is eindelijk zover, na 4 jaar promoveren en inmiddels een heleboel jaren verder ben ik blij en trots dat ik dit werk aan jullie mag aanbieden. Op de eerste dag van mijn promotietijd werd mij verteld dat dit de mooiste tijd uit mijn leven zou worden, en daar was niets aan gelogen. Ik ben ontzetten blij met iedereen die me in deze periode heeft geholpen, en wil in het bijzonder een aantal mensen bedanken.

Steven, mijn inspirator. Zelfs als we een telefoongesprek op 240km afstand hebben, voel ik je energie. Toen je me voorstelde om geen schildercursus in Italië te gaan doen maar bij jou onderzoek in Londen op te starten, zag ik het in eerste instantie niet zo zitten om naar die 'grijze' stad te gaan. Ik kon het niet méér bij het verkeerde eind hebben. Londen was één groot avontuur door jou, onze gesprekken, koffiemomenten en baravonden. Wat heb ik daarna veel heimwee gehad, maar gelukkig ging onze samenwerking door. Dank voor je vertrouwen. Jouw onuitputtelijke inzet en enthousiasme voor het leven zijn aanstekelijk. Er is niemand zoals jij, en ik ben blij dat ik op je pad heb mogen komen.

Lieve Proffie, mijn beschermengel. Bedankt voor alle mooie momenten samen. Uw lieve woorden, zachtaardigheid en altijd aanwezige glimlach maakten dat ik graag binnenliep voor een wijs advies of promotie-update. Uw harde werkmentaliteit en bourgondische instelling zijn typerend voor uw karakter. Dank dat u mij stimuleerde om uit te vliegen, ik denk aan u.

Beste Frank, mijn corrector. Je hebt voor de studies in dit proefschrift altijd gestreefd naar het maximale, en ik was meteen onder de indruk van jouw proza. Ik moest even wennen aan de rode pen, maar we hebben samen onze weg kunnen vinden en ik ben ervan overtuigd dat jouw nauwkeurigheid dit werk naar een hoger niveau heeft getild. Dank voor al je input en feedback!

Geachte Prof. van der Horst, Prof. Shiri-Sverdlov, Prof. Buhre, Dr. de Boer en Dr. Erdmann. Hartelijk dank voor het beoordelen van mijn proefschrift en uw deelname in de promotiecommissie, ik waardeer het zeer. Beste leden van de gehele promotiecommissie, dank voor uw deelname en uw bereidheid om met mijn van gedachten te wisselen op 8 juli.

Top HPB-club, dank voor jullie fijne samenwerking. Initieel in mijn rol als HPB-assistent, en daarna als promovendus hebben we veel patiënten begeleid en geïncludeerd maar ook veel mooie momenten daarbuiten gehad. Werken en op congres gaan met jullie was een feest!

Chère Prof. Leclercq, merci de m'avoir donné l'opportunité de faire des recherches dans votre laboratoire. Un lieu particulièrement inspirant, où vous dirigez votre groupe de recherche avec brio. Merci pour les souvenirs chaleureux.

Lieve oud-promotiecollega's (Anne-Claire, Martine, Frans, Junfang, Lori, Givan, Joyce-Manyi, Kiran, Audrey, Cathy, Lieke, Dennis, Kirsten, Dirk, Claire, Tim, Elwin, Milou, Selwyn, Cathelijne, Jacqueline, Jasper, Rob, Victor, Stefan, Edgar, Leontine, Inca, Ruben, Simon, Britt, Marissa, Kaatje, Sander, en labhelden Bas, Mo, Annemarie en Hans) van de heelkunde, waar moet ik beginnen? Eindeloze herinneringen hebben we gemaakt, en wat hebben we gelachen tijdens alle lunches, uitjes, fietstochten, quizzen in de Edds en borrels in de Thembi. We waren in onze promotiejaren een hechte groep, heel bijzonder om dat te mogen meemaken. Lief en (promotie)leed deelden we met elkaar, evenals de allerslechtste labgrappen. Onze generatie was toch echt de leukste ;)

Lieve Irene Fleur we leerden elkaar pas wat later in ons PhD-traject kennen maar ons contact was meteen zo fijn, ik ben heel blij dat we contact zijn blijven houden. Als ik ooit bij een chirurg moet komen, dan vraag ik naar jou! Toine, professor van de retrospectieve studies, mijn eerste HPB-momenten waren met jou. Dank dat je me wegwijs hebt gemaakt. Ik hou van je humor en ben supertrots op je.

Liliane, prachtige parel. Je nam mij tijdens onze promotietijd onder je vleugels in Londen en liet me kennismaken met (de kroegen van) de stad. Onze vriendschap begon in Charlotte Street Hotel met een fles Chileense wijn, en word sindsdien gekleurd door heerlijke avonden in het park, in de tuin of weekendjes terug naar 'onze' plek. Nog altijd gaat dat gepaard met zo'n zelfde glas stevige, rode druivensap. Ondanks dat je nu in Ghana woont en samen 'promoveren' in het Volkshotel in Amsterdam er niet meer in zit, weet ik zeker dat onze vriendschap zal blijven bestaan. Ik kom snel naar je toe!

Lieve vriendinnen van mijn eerste onderwijsgroep, oud-roomies, Missdaad en iedereen daarbuiten. Dank voor al jullie betrokkenheid en gezelligheid, jullie maken het leven mooier. Wat hebben jullie vaak mijn ontwijkende antwoord moeten aanhoren over 'hoe het nu staat met mijn proefschrift'. Op naar nog vele nieuwe herinneringen, borrels, en zonnige momenten samen!

Lieve collega's van de dermatologie, bedankt voor jullie steun en gezelligheid. Jullie maken dat ik iedere dag met veel plezier naar mijn werk ga. Vanaf het eerste moment voelde ik me thuis bij jullie, en dat gevoel is nooit weggegaan. Wat een geluk dat ik mijn opleidingsjaren samen met jullie mag delen.

Lieve David, mijn beste Phd-maatje. Jij hebt mijn 4 promotiejaren gemaakt tot de mooie tijd die het was, en me geleerd altijd 'waarom' te vragen. Vanaf onze ontmoeting op de heelkunde gang tot aan mijn afscheidsborrel destijds waren we onafscheidelijk. Jij initieerde alle lunches, organiseerde de culturele tour tijdens de congressen en nam ons mee op sleeptouw naar Italië om te fietsen. Als ik ergens mee zat kon ik altijd bij je terecht, en als ik hangry was trok je me mee naar de snoepautomaat. Naast het sociale dier dat je bent en je heelkunde opleiding, leiden jij en Sophie ook nog eens jullie prachtige gezin. Zo knap hoe jullie dat allemaal weten te combineren, jullie zijn een voorbeeld voor me. Ik mis onze avonden Amazing Oriental en eindeloos koken, maar ben blij dat we ondanks de afstand nog altijd in elkaars leven zijn. Ik zie uit naar meer mooie momenten in Maastricht en Den Haag!

Lieve Willemijn, mijn rots in de branding. Alweer bijna 20 jaar zijn we in elkaars leven, we kennen elkaar door en door en ik kan me geen betere vriendin wensen. Altijd sta je voor me klaar met lieve woorden, een appje of een kaartje. Ik ben zo trots hoe jij en je mooie gezin in het leven staan! Jij hebt me geleerd het leven te vieren. De herinneringen zijn nu al ontelbaar, maar er komen er nog zoveel bij. Ik wens dat we, als we heel veel jaren en grijze haren verder zijn, samen broodjes kroket achter de geraniums eten.

Lieve papa, mama en Tom. Wat voel ik me gezegend dat ik onder jullie hoede heb mogen opgroeien. Jullie zijn de sterkste mensen die ik ken. Niks is te veel voor jullie, en ik ben ontzettend dankbaar dat jullie altijd voor me klaar staan. Mama, jij weet altijd precies de juiste gevoelsreflecties te geven, waarna papa met de beste praktische oplossing voor het probleem komt. Bruti, in onze jongere jaren zo verschillend maar stiekem lijken we ontzettend op elkaar (of gewoon steeds meer?), ik ben zo trots op je. ~~Mijn werkethos heb ik van~~ Jullie werkethos is ongeëvenaard, al ben ik minstens zo blij om jullie buiten het werk om zo te zien genieten van het leven. Ik koester onze sterke band, laten we allemaal nog lang gezond blijven.

Sayang Hanum, betapa menyenangkannya kamu di sisiku hari ini. Kita belum saling kenal selama itu, tapi kamu membuat hidupku jauh lebih menyenangkan. Saya menantikan semua petualangan yang akan kita jalani bersama, saya suka kamu!

CURRICULUM VITAE

Kim van Mierlo werd op 6 mei 1988 geboren in Veldhoven (Nederland). In 2006 slaagde ze voor het gymnasium op het Pleincollege Van Maerlant in Eindhoven. Zij verhuisde daarna naar Maastricht voor de opleiding Geneeskunde en de minor Gezondheidsrecht aan de universiteit aldaar, welke zij afrondde in 2012. In het laatste half jaar van haar opleiding volgde zij haar wetenschappelijke stage op het gebied van leverfalen na leverchirurgie (bij Prof Rajiv Jalan, Liver Failure Group) aan University College London, waar de basis werd gelegd voor haar promotieonderzoek. Na een half jaar als basisarts op de afdeling hepatopancreatobiliaire chirurgie te hebben gewerkt, startte ze met haar promotieonderzoek bij NUTRIM (School of Nutrition and Translational Research in Metabolism) aan de Universiteit Maastricht hetgeen heeft geresulteerd in de huidige thesis. Na vier jaar onderzoek aan deze universiteit met tevens onderzoeken in de Université Catholique de Louvain (UCL; Brussel) en Universitätsklinikum Aachen (Aken), besloot zij in 2017 een andere richting in te slaan en startte als arts-assistent niet in opleiding tot dermatoloog op de polikliniek dermatologie in het Catharina Ziekenhuis te Eindhoven. Na enkele maanden klinisch werk te hebben verricht werd zij aangenomen voor de opleiding dermatologie in het Leids Universitair Medisch Centrum onder leiding van dr. A. Lavrijsen, met als aandachtspunt de anogenitale dermatosen. Zij rondt de opleiding tot dermatoloog af in maart 2023.