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Colorectal Cancer Liver Metastases

Effects of Chemotherapy on Liver Parenchyma and Resections

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Abstract
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Current multimodal treatment of colorectal cancer liver metastasis often combines liver resections with preoperative chemotherapy with a 5-year survival of 40-50%. Preoperative chemotherapy includes conversion of initially non-resectable situation and control of micrometastatic disease. Despite its potential advantages also problems with associated steatosis, steatohepatitis and sinusoidal injury has been discussed. **Paper I** focused on prospective steatosis evaluation prior to resections using proton MR spectroscopy, most sensitive non-invasive method. Proton MR spectroscopy showed high concordance with digital quantification of steatosis and was also able to predict steatohepatitis with 100% sensitivity and 89% specificity without knowing lobular inflammation or hepatocyte ballooning. **Paper II** focused on portal vein hemodynamics changes in patients treated with oxaliplatin-based treatment and with sinusoidal injury. Magnetic resonance imaging flowmetry demonstrated portal vein dilatation associated with oxaliplatin treatment. Patients with SI showed a tendency towards decreased mean portal flow velocity. Portal vein flow was not changed. This may indicate that SI is associated with an increased resistance to blood flow in the liver parenchyma and stasis in splanchnic system. **Paper III** attempted to enlighten the effects of FOLFOX treatment on human liver tissue 6 weeks after treatment cessation by quantification of protein expression changes using label-free global proteome analysis. Deep proteome analysis identified 5891 proteins, where machine learning algorithm identified 3% of classifying proteins, associated with changes in DNA replication through upregulation of the minichromosome maintenance complex and with the innate immune response. Significant changes were observed in 1% of proteins, associated with DNA replication and cell cycle entry. Results support the hypothesis that liver has already regenerated from the FOLFOX treatment injury after 6 weeks. **Paper IV** aimed to identify possible patient, disease and chemotherapy characteristics associated with liver specific and severe general complications in a retrospective single centre cohort composed of 516 consecutive resections. Chemotherapy with more than 4 cycles of oxaliplatin was associated with post-hepatectomy hemorrhage. Underlying liver disease and diabetes mellitus were associated with 90-day mortality. Size of resection, intraoperative blood loss and transfusions were verified as independent predictors of liver specific complications to resections.

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"Our greatest weakness lies in giving up. The most certain way to succeed is always to try just one more time."

Thomas A. Edison

To my family...

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I Urdzik, J., Bjerner, T., Wanders, A., Weis, J., Duraj, F., Haglund, U., Norén, A. (2012) The value of pre-operative magnetic resonance spectroscopy in the assessment of steatohepatitis in patients with colorectal liver metastasis. *Journal of Hepatology*, 56(3):640-646

II Urdzik, J., Bjerner, T., Wanders, A., Duraj, F., Haglund, U., Norén, A. (2013) Magnetic resonance imaging flowmetry demonstrates portal vein dilatation subsequent to oxaliplatin therapy in patients with colorectal liver metastasis. *HPB*, 15(4):265-272

III Urdzik, J., Vildhede, A., Wisniewski, J., Duraj, F., Haglund, U., Artursson, P., Norén, A. (2014) Global proteome changes in liver tissue 6 weeks after FOLFOX treatment of colorectal cancer liver metastases. *Manuscript*

IV Urdzik, J., Haglund, U., Duraj, F., Norén, A. (2014) Association between patient and chemotherapy characteristics with short-term outcomes of resections for colorectal cancer liver metastases. *Manuscript*

Contents

Abbreviations

APRI	aspartate aminotransferase to platelet ratio
AUC	area under receiver operating characteristic curve
BL	biliary leakage
BMI	body mass index
CASH	chemotherapy-associated steatohepatitis
CRCLM	colorectal cancer liver metastases
CVI	combined vascular injury
DQS	digital quantification of steatosis
FDR	false discovery ratio
FLv	fluorouracil leucovorin
FOLFOX	fluorouracil leucovorin oxaliplatin treatment
IR	irinotecan-based chemotherapy
ISGLS	International Study Group of Liver Surgery
LC-MS/MS	liquid chromatography – tandem mass spectrometry
NAFLD	non-alcoholic fatty liver disease
NAS	non-alcoholic fatty liver disease activity score
NASH	non-alcoholic steatohepatitis
MCM	minichromosome maintenance complex
MR	magnetic resonance
MRIF	magnetic resonance imaging flowmetry
noOX	without oxaliplatin-based chemotherapy
OX	oxaliplatin-based chemotherapy
OX+noSI	with oxaliplatin-based chemotherapy without sinusoidal injury
OX+SI	with oxaliplatin-based chemotherapy with sinusoidal injury
PCA	principal component analysis
PCI	portal chronic inflammation
PHH	post-hepatectomy hemorrhage
PHLF	post-hepatectomy liver failure
PVE	portal vein embolization
RFE-SVM	recursive feature elimination – support vector machine
ROC	receiver operating characteristic
ROS	reactive oxygen species
SI	sinusoidal injury
VEGF	vascular endothelial growth factor
^{1}H MRS	proton magnetic resonance spectroscopy

1 Introduction

Colorectal cancer is the third most common cancer worldwide and the incidence in Sweden is just under 6000 new cases per year. The ratio between colon and rectal cancer is 2:1. Liver is the organ most commonly affected by metastases, with an incidence of around 25%. Colorectal cancer liver metastases (CRCLM) are diagnosed synchronously with the primary tumor in about 15% of patients and another 8–15% are diagnosed at follow up within five years of colorectal cancer surgery (1). The risk of CRCLM increases with higher primary tumor TNM stages; 4% for stage I, 13% for stage II and 30% for stage III (1).

The introduction of new chemotherapeutic agents has altered the management of patients with CRCLM. A multidisciplinary approach plays a key role in this multimodal treatment. Preoperative chemotherapy primarily allows the conversion of non-resectable disease to resectable (2) and the control of micrometastatic disease (3, 4). This strategy of combining liver resection with preoperative chemotherapy has achieved a 5-year survival of 40–50% (5-9). Currently, approximately 75% of patients at our hospital receive chemotherapy prior to resection for CRCLM. Although there are obvious potential advantages with preoperative chemotherapy, several drawbacks have come to light. Attention has particularly focused on the problem of chemotherapy-associated toxicity on non-tumorous liver parenchyma with specific patterns of histopathological damage (10) and a possible association with postoperative complications.

2 Background

2.1 Preoperative chemotherapy for colorectal cancer liver metastases

Patients with initially non-resectable CRCLM can be offered resection following chemotherapy and a subsequent reduction in tumor size (2), providing overall survival benefits from radical liver resection in about a third of patients (8). The object of preoperative chemotherapy in patients with primary resectable CRCLM is to control micrometastatic disease and suppress the biological activity of the cancer. Treatment response assessment enables the identification and selection of patients with favorable tumor biology (11). This approach prolongs disease-free survival (3, 4), but not overall survival (8). Oxaliplatin-based chemotherapy (OX) is preferred as first-line treatment, and irinotecan-based chemotherapy (IR) is often used second-line in treatment response failure. On the other hand, complete radiological tumor response makes locating tumors intraoperatively more difficult (12), and any remaining viable tumor cells at the time of surgery may lead to incomplete resection and early recurrence (13). Another problem is disease progression during neoadjuvant chemotherapy in 5–37% patients, in whom different treatment strategies may be applied (8). Prolonged exposure to OX has been associated with chemotherapy resistance and aggressive tumor behavior (14).

2.2 The effects of preoperative chemotherapy on liver parenchyma

Several adverse effects of preoperative chemotherapy on non-tumorous liver parenchyma have been described: steatosis in patients treated with fluorouracil-leucovorin (FLv), which is the main compound used in modern chemotherapy, chemotherapy-associated steatohepatitis (CASH) (15) following IR, and sinusoidal injury (SI) following OX injury (16-18).

Steatosis and steatohepatitis

Steatosis and steatohepatitis form the histopathological basis of the surgical term "yellow liver syndrome". Non-alcoholic fatty liver disease (NAFLD) is defined as histopathological liver steatosis of more than 5%. The full spectrum of NAFLD is characterized by steatosis, lobular inflammation and hepatocyte ballooning. NAFLD can develop further into non-alcoholic steatohepatitis (NASH). The level of activity can be assessed by a complex, reliable and validated pathological diagnostic system called the NAFLD activity score (NAS), as proposed by the NASH Clinical Research Network (19). Lately, portal chronic inflammation (PCI) has also been proposed as a marker of advanced NAFLD (20).

Steatosis has been observed in patients treated with FLv (21), but a causative association remains unproven and the possible mechanism is unclear. FLv is associated with mitochondrial membrane disruption, which is followed by a reduction in membrane potential which may lead to impaired fatty acid oxidation and the subsequent accumulation of reactive oxygen species (ROS) (22). FLv is also associated with microsomal cytochrome P450 production of ROS. FLv metabolites such as fluoro-β-alanine may reduce the hepatocyte's capacity to metabolize other drugs (23). The prevalence of histopathologically confirmed steatosis ranges from 6 to 98% in patients treated with preoperative chemotherapy (8). An alternative or additive explanation for chemotherapy-related steatosis in patients with CRCLM is the rising prevalence of obesity and metabolic syndrome in the general population (24).

Steatohepatitis caused by chemotherapy is known as CASH, but its pathogenesis remains unclear. Steatohepatitis following IR has been reported (15). One plausible model is the "two-hit model", whereby CASH only develops in liver parenchyma that is steatotic due to pre-existing obesity or metabolic syndrome, and in which IR acts as the second hit. The molecular basis is, again, not clearly defined. The lipophilic irinotecan metabolite SN-38 is inactivated by microsomal cytochrome P450 enzymes (25). Similar lipophilic molecules accumulate in mitochondria, inhibiting oxidation and electron transfer along the respiratory chain and producing ROS (26). The "two-hit model" may also explain the variation in the reported incidence of steatohepatitis. Ryan et al could not establish an association between chemotherapy and steatohepatitis with an observed incidence of only 2.4% in the studied group of 334 patients. Steatosis and steatohepatitis were only associated with body mass index (BMI) >30 kg/m^2 (16). On the other hand, other investigators have observed an association between chemotherapy and steatohepatitis with a prevalence of 8% (27) and between IR and steatohepatitis with a prevalence of 2% (28). Another study observed a prevalence of steatohepatitis of up to 58%, but could not demonstrate an association with any specific chemotherapy regimen (29).

Sinusoidal injury

"Blue liver syndrome" (the term relates to its typical spotty bluish liver surface) is the clinical expression for SI associated with OX. The prevalence of SI after OX treatment ranges from 5% (27) to 50% (17, 18). The probable reason for this wide range is the lack of a uniform classification and limited awareness among pathologists of the full spectrum of histopathological changes (16). Initially, SI was described as sinusoidal dilatation (17), but further research revealed a full spectrum of histopathological changes including congestion and hemorrhage leading to hepatocyte loss called parenchymal extinction lesion, perisinusoidal and centrilobular fibrosis, sinusoidal obstruction, nodular regenerative hyperplasia and possibly veno-occlusive disease-like changes (18). Intra- and inter-observer variation can be minimized by a semi-quantitative scoring system for combined vascular injury (CVI) (16). Some patients develop SI after only a short period of treatment whilst others do not develop SI even after prolonged treatment. This has given rise to the hypothesis of individual susceptibility to OX-induced injury (30). This hypothesis is supported by the association between SI development and polymorphisms in the nucleotide excision repair genes ERCC2 (31), copper transporter ATP7B (32) and glutathione S-transferase M1 (33).

The exact molecular pathway behind the effects of oxaliplatin on liver parenchyma remains unclear. Microarray studies attempting to provide an overview of the whole range of changes associated with SI in humans suggest that angiogenesis, cellular adhesion and extracellular matrix components (30, 34) are involved, along with activation of the acute phase response, the coagulation system, hepatic fibrosis and hypoxic factors (34).

Generation of ROS in sinusoidal endothelial cells is one of the important mechanisms. It causes depletion of glutathione (35, 36) and decreased redox potential (37, 38). Along with actin dissociation in the cytoskeleton (39), it upregulates activity and expression of metalloproteinases such as MMP9. Biochemical changes initially lead to rounding up of sinusoidal endothelial cells, followed by detachment of endothelial cells from the perisinusoidal space (40). Red blood cells begin to penetrate into the space of Disse, which leads to dissection of the sinusoidal lining, downstream sinusoidal embolization and obstruction of sinusoidal flow (41), observed as sinusoidal dilatation (40). Larger hemorrhages into the hepatocyte plates are followed by their disruption, leading to parenchymal extinction lesions. These lesions has always been associated with hepatic vein injury with intimal edema, intimal or full vein wall hemorrhage, intimal fibrosis and lumen obstruction further compromising hepatic venous outflow (16). Later, these changes proceed to hepatocyte loss, perisinusoidal and centrilobular fibrosis and finally to end-stage nodular regenerative hyperplasia (18).

Pro-thrombotic changes in injured sinusoids suggest another mechanism for SI. Elevation of plasminogen activator inhibitor-1 (SERPINE1) (42),

important in extracellular matrix remodelation and hepatic vein thrombosis (43) and Von Willebrand factor (30) have been documented following OX treatment. However, their increased expression, as well as the matrix remodeling pattern, can also be explained by the presence of CRCLM itself (44). The role of thrombosis in SI pathophysiology can be inferred from the clinical finding that aspirin reduces the risk of SI in patients treated with OX (45).

Finally, the role of angiogenic and vasoactive factors in the pathogenesis of SI has been documented. Endothelin-1 mediates sinusoidal constriction and increases resistance to venous flow (38). Vascular endothelial growth factor (VEGF) leads to a hyperdynamic splanchnic circulation and portal hypertension (46). VEGF induces MMP-9 expression in sinusoidal endothelial cells studied in a rat model (37). The importance of VEGF is supported by the findings of several clinical studies that have shown that anti-VEGF antibodies (bevacizumab) administered together with OX treatment protect against the development of SI (18, 47, 48).

2.3 Assessment of chemotherapy-induced liver parenchyma changes before surgery

There is no agreement as to which tests should be performed preoperatively to evaluate liver function reserve (49). Co-morbidities and the use of preoperative chemotherapy increase the importance of liver remnant "quality" assessment. Both CASH and SI may be present in the absence of any clinical manifestation of hepatotoxicity during or after completed therapy (50). Several studies have attempted to identify predictive factors for SI and CASH, but an accurate, non-invasive method of assessment has yet to be found.

Classical histopathology analysis of resected non-tumorous liver parenchyma is still the gold standard among the diagnostic alternatives, despite strong observer-dependency and low reproducibility (51). Preoperative liver parenchyma biopsy is invasive and not recommended due to small tissue amounts obtained and possible sampling error (10). Digital quantification of steatosis (DQS) using computerized image analysis of scanned histopathological slides (51, 52) increases the objectivity of histopathology, but a tissue sample is still needed.

Conventional ultrasound and computer tomography do not permit accurate identification of hepatic steatosis (53, 54) and are unable to identify steatohepatitis. Methods based on magnetic resonance (MR) (54), particularly proton MR spectroscopy (^{1}H MRS), are currently considered the most sensitive non-invasive methods of detecting liver steatosis (55).

Several studies have tried to identify predictive factors for SI (45, 56-59). Laboratory parameters such as raised gamma-glutamyl transpeptidase levels

of 1.5× normal (45) or an aspartate aminotransferase to platelet ratio index (APRI) of >0.36 (58) can be used to predict SI preoperatively. APRI is also reported as a predictor of risk for OX-induced splenomegaly, even before the start of chemotherapy (60). Increased spleen size has been reported following OX (61) and an increase in spleen size of ≥50% during OX identifies patients at high risk of SI (57). A hypothesis that OX-induced SI can lead to portal hypertension and subsequently splenomegaly and thrombocytopenia has been put forward (62). Two case reports showed that OX may induce significant portal hypertension verified by direct measurement of portal venous pressure (62, 63). Portal flow hemodynamics can also be evaluated noninvasively by Doppler ultrasonography or by the more objective and reproducible technique of magnetic resonance imaging flowmetry (MRIF) (64). Superparamagnetic iron oxide enhanced MR imaging has been used to visualize SI in the liver, but the method showed wide discrepancy in SI prediction sensitivity, 14% and 87% (56, 59).

2.4 Clinical relevance of preoperative chemotherapy effects on liver parenchyma

Postoperative complications of liver resection depend on several factors. The extent of metastatic spread and the size of the liver resection (65) needed to obtain cancer-free status along with related intraoperative blood loss are well-known and substantial risk factors (66). Preoperative chemotherapy-induced liver parenchyma injury may influence regeneration after resection and also the clinical results and complications of surgery.

Moderate or severe liver steatosis (>30% steatosis according to histopathology), found in patients with a high BMI, is associated with complicating infection, but not with severe morbidity or mortality (67). Liver steatosis caused by co-morbidities such as diabetes mellitus and obesity is a known risk factor in liver surgery (68). Vauthey et al reported that simple steatosis is not associated with increased postoperative morbidity or mortality after liver surgery (27). Other investigators have found that steatosis is associated with increased intraoperative blood loss, raised postoperative complication rates and a longer intensive care unit stay, but 26% of patients with steatosis also had a BMI of >30 kg/m^2, a known risk factor itself (69).

Initial reports showed an alarming increase in 90-day mortality (up to 15%) due to liver failure following resection in patients with CASH associated with IR treatment (27, 45). However, other authors cast doubt on this. Ryan et al found CASH to be uncommon and instead found that SI was a common adverse effect of preoperative chemotherapy, neither having any impact on postoperative outcome (16).

Others have shown that SI is associated with a greater need for perioperative blood transfusion (70), increased postoperative morbidity (3, 29, 48, 71, 72), and possibly has a negative impact on long-term prognosis (73). An association has been documented between SI and postoperative morbidity following intense chemotherapy with a high number of chemotherapy cycles or several lines of chemotherapy (29, 48, 71). However, other studies, despite an identified association between OXC and SI, have not been able to show an increase in postoperative morbidity or mortality in properly selected patient cohorts (28, 74, 75).

The ability of the liver to regenerate after resection depends on the volume of liver remnant as well as on whether adequate blood inflow, outflow and biliary drainage have been preserved. Volumetric analysis estimates actual or standardized future liver remnant ratio, and this correlates with the postoperative incidence of complications, particularly of liver failure. Future liver remnant ratio under a threshold of 25% is associated with an increased risk of postoperative liver failure in patients without underlying liver disease (76-79). In patients intensively treated with preoperative chemotherapy for CRCLM, the "safe" threshold of future liver remnant ratio needed to prevent postoperative liver failure was estimated at 38% (80). Portal vein embolization (PVE) is used as a future liver remnant augmentation technique. PVE partially mimics post resectional hyperperfusion of the liver remnant and can be viewed as a biological test of liver regeneration capability (81). Narita et al demonstrated that OX-related SI inhibits future liver remnant hypertrophy following PVE and induces postoperative liver failure (82). A possible explanation lies in the hemodynamic changes seen in SI when compromised venous outflow leads to increased parenchymal blood flow resistance resulting in relatively reduced future remnant hyperperfusion post PVE and resection. In contrast, Giraudo et al observed only delayed hypertrophy after PVE (83), and other investigators found that preoperative chemotherapy had no effect on future liver remnant hypertrophy after PVE (84-86). These studies, however, did not analyze the extent and severity of chemotherapy-related changes in non-tumorous liver parenchyma. These studies suggest that liver regeneration is impaired after intensive preoperative chemotherapy, mainly in initially non-resectable patients.

The time interval between chemotherapy and liver resection seems to be an important factor affecting postoperative outcome, with the risk of postoperative complications increasing if the interval is ≤4 weeks (87), while others question this factor (75). The indocyanine green test, used as a measure of hepatic functional reserve, improves during the 2-4 weeks period after cessation of chemotherapy, especially in patients who have received ≥6 cycles of preoperative chemotherapy (88). It remains unclear how long chemotherapy-induced changes persist after treatment. An increase in spleen size of ≥50% during OX treatment, which strongly correlates with SI, remained 6 months after OX cessation (57). Ryan et al observed SI in 11% of patients without

any chemotherapy within one year of liver surgery. However, chemotherapy carried out more than one year before surgery was not reported (16). Persistence or progress of SI between two-stage liver resection (4–7 week interval between operations) despite chemotherapy cessation suggests that prolonging the time to resection provides no further benefit from a surgical point of view (12).

All the above data suggest that it is unlikely that any single factor lies behind the high risk of postoperative complications following chemotherapy for CRCLM. A combination of risk factors may be a better predictor of the short-term outcomes of liver surgery for CRCLM.

3 Aims

The aim of this thesis was to investigate the effects of preoperative chemotherapy on liver parenchyma and on the clinical results in patients undergoing resection for CRCLM. The specific aims were:

Paper I

- To evaluate the diagnostic value and reliability of the clinical data and non-invasive ^{1}H MRS versus classical histopathology and DQS in assessment of steatosis and NAFLD activity in patients treated with chemotherapy prior to resection for CRCLM.

Paper II

- To investigate if portal vein flow hemodynamics, measured by MRIF, changes in patients with SI or OX treatment for CRCLM.

Paper III

- To elucidate the effects of FOLFOX treatment on human liver tissue as reflected in protein expression changes and their associations with biological processes and pathways.

Paper IV

- To identify if patient and chemotherapy characteristics are associated with liver-specific and general complications following resection for CRCLM.

4 Patients and Methods

The patient material and methods used have been fully described in the separate study reports (paper I–IV) to which the reader is referred for details. A brief summary is presented below.

4.1 Ethical considerations

The local ethics committee gave written approval for all studies. Written informed consent was obtained from each patient (paper I – III).

4.2 Patients and methods

Common patients and methods paper I and II

All patients planned for CRCLM resection after assessment by a multidisciplinary team at Uppsala University Hospital were prospectively evaluated for paper I between January 2007 and December 2009. A planned minimum two-segment resection providing sufficient amounts of non-tumorous liver tissue for histopathology was an obligatory inclusion criterion. General contraindications to MR examination were exclusion criteria. No patient meeting the inclusion criteria was excluded from the study provided the MR facility was available. The paper II cohort was a subgroup of paper I patients undergoing surgery between October 2007 and December 2009. ^{1}H MRS and MRIF were performed on the day before surgery and the results did not influence the prior decision to carry out liver resection.

Histopathology

Non-tumorous liver parenchyma samples were obtained immediately after surgery, by taking tissue blocks approximately 40×40×7 mm, trying to omit big vessels or duct structures and a minimum of 20 mm from the peritoneal liver surface, the resection margin and the metastases. These samples were immediately fixed in 10% neutral buffered formalin (4% formaldehyde), embedded in paraffin blocks, cut into 3 µm thickness and stained with hematoxylin & eosin and van Gieson. In addition, for paper II, GOS (Gordon and Sweet's) reticulin staining and immunohistochemical stainings with antibod-

ies against ki-67 were used. All samples were evaluated by one experienced liver pathologist blinded to all clinical data, DQS, ^{1}H MRS and MRIF results.

Steatosis was graded semi-quantitatively (19), Table 1. More than a mild steatosis (grade ≥2) was interpreted as clinically significant "marked" steatosis (67). NAS was used to evaluate NAFLD activity, Table 1 (19), and NAS of ≥4 points was interpreted as steatohepatitis (56). Fibrosis stages were recorded using a score adapted from Kleiner et al, Table 1 (19). PCI was assessed using the semi-quantitative scale proposed by Brunt et al (20), modified because of the increased size of slides compared to the originally described liver biopsies (0 = none, 1 = sporadic, 2 = more than sporadic). More than sporadic PCI was considered another marker of advanced NAFLD, unrelated to lobular inflammation and steatohepatitis (20).

Table 1. Non-alcoholic fatty liver disease activity score (NAS) and evaluation of fibrosis. NAS score is the unweighted sum of semi-quantitative sub scores for steatosis, lobular inflammation and hepatocellular ballooning with a possible range from 0 to 8.

Histopathological features		**NAS (0–8)**
Steatosis	≤5%	0
	5-33%	1
	33-66%	2
	≥66%	3
Lobular inflammation (per x200 field)	None	0
	≤2 foci	1
	2-4 foci	2
	≥4 foci	3
Hepatocellular ballooning	None	0
	few, mild ballooning	1
	prominent ballooning	2
Fibrosis	no fibrosis	0
	zone 3 perisinusoidal fibrosis (special fibrosis staining required to identify)	1a
	zone 3 perisinusoidal fibrosis (easily seen on haematoxylin & eosin)	1b
	periportal/portal fibrosis only	1c
	zone 3 with periportal/portal fibrosis	2
	as above with bridging fibrosis	3
	cirrhosis	4

In paper II, SI was graded according to the CVI score, Table 2. CVI ≥3 was recognized as clinically relevant SI (16).

Table 2. Combined Vascular Injury (CVI). CVI score is the unweighted sum of semi-quantitative and qualitative sub scores for associated histopathological features with a possible range from 0 to 13.

Histopathological features		Score (0–13)
Diffuse sinusoidal dilation	None	0
	<1/3 lobule	1
	1/3-2/3 lobule	2
	Entire lobule	3
Small vessel loss/obliteration	None	0
	<1/3 of vessels	1
	1/3-2/3 of vessels	2
	>2/3 of vessels	3
Focal hepatocyte plate disruption	None	0
	Present	1
Parenchymal extinction lesion	None	0
	Rare	1
	Frequent	2
Nodular regenerative hyperplasia	None	0
	Subtle	1
	Obvious	2
Peliosis	None	0
	Present	1
Veno-occlusive disease-like change	None	0
	Present	1

Additional methods paper I

Proton magnetic resonance spectroscopy

The single-voxel ^{1}H MRS was measured by an Achieva Philips 3 T scanner using STEAM sequence in free breathing. The net acquisition time was 2 min 24 sec. The volume of interest $30\times30\times30$ mm^3 was placed in non-tumorous liver parenchyma, trying to omit large blood vessels and ducts, as centrally in the parenchyma as possible.

Digital quantification of steatosis

DQS was performed using an image analysis technique. One haematoxylin & eosin stained slide from each patient was scanned and also used for the pathologist's evaluation. ImageJ software was used for digital image analysis by creating an automatic macro with the possibility of manual correction. Only macrovesicular steatosis with droplets $\geq$6.98 μm^2 was considered, with the intention to exclude potential error by including sinusoids, small bile

ducts and blood vessels which can be incorrectly graded as “microsteatosis” by automated analysis (89).

Additional methods paper II

Magnetic resonance imaging flowmetry

The patients were examined in the supine position by MRIF using the same scanner with a SENSE-CARDIAC coil. Axial and coronal steady-state free precession images of the portal venous system were used to localize the correct orientation. Cine phase-contrast imaging was performed during a breath hold using triggering by vector electrocardiogram with an acquisition time of 18 sec. Using Philips software, the cine phase-contrast flow rates were calculated by integrating the velocity product of 17 velocity images spanning the cardiac cycles. Mean flow rates were derived from the average flow rate during each phase of the cardiac cycle and portal flow, cross-section area, mean and peak velocities were calculated. The radiologist was blinded to clinical and histopathology data.

Patients and methods paper III

Liver tissue samples were obtained from patients undergoing a major liver resection for CRCLM at Uppsala University Hospital between 2009 and 2012. The non-tumorous liver tissue samples were cut immediately in the operation room as soon as the liver specimen had been removed from the patient, and immediately stored at −80 ºC until further proteomic analysis could be carried out.

Global proteomic analysis

Relative protein quantification of the non-tumorous liver tissue samples was analyzed as described (90-93) in paper III. Briefly, pieces of thawed human liver tissue were homogenized and lysed. Each lysate was processed and analyzed in duplicate. Cleavage of proteins was carried out by a consecutive two-step digestion with endoproteinase LysC and trypsin (94). The digests were loaded on SAX microcolumns at pH 11 (93), the peptides were eluted with a buffer of pH 2 and organic solvent was evaporated. The peptide fractions were analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) using 4 h linear acetonitrile gradients for the reverse phase chromatography and QExactive instrument for mass spectrometry (95). The spectra were searched using the “Andromeda search engine” (96) and analyzed using the MaxQuant software version 1.2.6.20 using the “matching between runs” option (97). Protein signal intensities were normalized by log2 transformation and used as a measure of abundance.

Patients and methods paper IV

All consecutive patients operated with liver resection for CRCLM at Uppsala University Hospital from January 2000 to December 2013 were identified from the prospective clinical database. Our institution is a referral center for liver surgery of 1.4 million inhabitants. The study population comprises 516 liver resections in 471 patients.

Patient characteristics, extent of liver metastases and treatment data were obtained from the local prospective database and double checked by systematic review of medical records. Any missing information was obtained from the local medical record systems or from regional oncology departments. Comorbidities were evaluated according to the Elixhauser comorbidity score (98) and patients were divided into two groups based on the median. Patients were also divided into older and recent half according to operation date, in order to identify changes in the treatment with time. All consecutive preoperative chemotherapy regimens were recorded: type, number of cycles and time from last day of therapy to liver surgery. Chemotherapy was defined as given ever, within 1 year, 6 months and 30 days before liver resection.

Liver-specific complications after liver surgery were defined (Table 3) and graded according the International Study Group of Liver Surgery (ISGLS) as post-hepatectomy hemorrhage (PHH) (99), biliary leakage (BL) (100), post-hepatectomy liver failure (PHLF) (101). Severe general complications were defined according Dindo-Clavien classification of surgical complications as grade IIIb or larger (102), and 30- and 90-day mortality were counted.

Table 3. International Study Group of Liver Surgery definitions of liver specific complications.

	ISGLS definition	Grade	
PHH	drop in haemoglobin level >3 g/dl post-operatively compared with the post-operative baseline level and/or any post-operative transfusion of packed red blood cells for a falling haemoglobin and/or the need for radiological intervention (such as embolization) and/or re-laparotomy to stop bleeding	**A**	transfusion of ≤2 units of packed red blood cells
		B	transfusion of >2 units of packed red blood cells
		C	need for invasive re-intervention such as embolization and/or re-laparotomy
BL	bilirubin concentration in the drain fluid at least 3 times the serum bilirubin concentration on or after postoperative day 3 or as the need for radiologic or operative intervention resulting from biliary collections or bile peritonitis	**A**	no change in patients' clinical management
		B	active therapeutic intervention but is manageable without re-laparotomy
		C	re-laparotomy is required
PHLF	impaired ability of the liver to maintain its synthetic, excretory, and detoxifying functions, which are characterized by an increased international normalized ratio and concomitant hyperbilirubinemia (according to the normal limits of the local laboratory) on or after postoperative day 5	**A**	no change of the patient's clinical management
		B	clinical management deviates from the regular course but does not require invasive therapy
		C	need for invasive treatment

4.3 Statistics

Basic Statistics

Data were analyzed using statistical package software SPSS® Statistics (IBM, USA) or the current version of R software (http://www.r-project.org/). In descriptive statistics, nominal variables were expressed by number and proportion percentage, differences demonstrated by Fisher exact test or Pearson Chi-square test when appropriate. Continuous variables were expressed by median and interquartile range or mean and standard deviation. Differences were demonstrated by the Mann-Whitney or Kruskal-Wallis tests when appropriate. Multiple comparison tests p-values were adjusted according to Bonferroni correction. Associations between quantitative variables were tested by Spearman correlations. Two-sided p-value <0.05 was considered statistically significant.

A Bland-Altman assessment for agreement was used to compare quantitative DQS and ^{1}H MRS and interclass correlation coefficients for rescored quantitative methods against semi-quantitative histopathology in paper I. The receiver operating characteristic (ROC) method was used to evaluate, by univariate analysis, identified predictors of marked steatosis and steatohepa-

titis in paper I and hemodynamic variables and their combination in SI prediction in paper II. Area under receiver operating characteristic curve (AUC), sensitivity and specificity and their 95% confidence intervals (95% CI) were calculated.

Proteome data analysis

Proteome data in paper III were analyzed using Perseus software (http://www.perseus-framework.org). To assess intra- and inter-individual proteome variability, unsupervised hierarchical clustering (on z-scored data) according to average Euclidean distance and principal component analysis (PCA) were performed for all the technical replicates. Classifying proteins were identified as the smallest set of high ranked proteins achieving minimal classification error rate using Recursive Feature Elimination – Support Vector Machine (RFE-SVM) and cross-validated by the leave-one-out method. Differently expressed proteins were identified using Welch t-test corrected for false discovery ratio (FDR) <5% counted by permutation method with 5000 runs and manually tuned exchangeability factor s_0. The method prevented influence of possible technical bias by strictly omitting technical replicates from permutations counting (103). The lists of classifying and relevantly changed proteins were analyzed for physical and functional associations using STRING 9.1 (http://string-db.org). Connections were expressed as a sum of functional, evidence and action scores. Overlaps with gene ontology categories and pathways were expressed using FDR adjusted p-value.

Multiple logistic regression

In paper IV were univariate associations between all characteristics and outcomes analyzed by simple logistic regression (variables with a p-value <0.1 were entered into a multivariate logistic regression model). Stepwise selected variables were included in the final model if they had a p-value of <0.05. Risk was estimated as odds ratio (OR), its 95% CI, and relative risk (RR) compared to the whole group was calculated. If comorbidities or chemotherapy characteristics were not included in the final model, their modulating effect was analyzed by entering them into the second layer of the logistic regression model.

5 Results

5.1 Paper I

Appropriate liver tissue samples together with ^{1}H MRS examination were obtained in 36 patients. One patient identified to be an outlier on Bland-Altman diagram of ^{1}H MRS and DQS was excluded from analysis due to a suspected sampling error. 71% patients received preoperative chemotherapy, mainly OX in combination with FLv or Xeloda. Five patients had second-line therapy. A median of 6 cycles (5–7) were given and the interval between chemotherapy cessation and liver surgery was 6 weeks (3–13).

80% of the patients had liver steatosis defined as >5% by the pathologist. Marked steatosis (grade 2 or more) was observed in 26% patients, who had significantly higher weight ($p=0.039$) and BMI ($p=0.005$). 78% of the patients with marked steatosis had received preoperative chemotherapy, but no significant associations were observed with comorbidities, type and number of cycles or time to surgery after chemotherapy cessation. Seven patients (20%) had marked steatosis and NAS ≥4 indicating steatohepatitis. They had a significantly higher BMI than patients with NAS <4 ($p=0.002$). Five of these seven patients with steatohepatitis had received preoperative chemotherapy. No other associations in the data on chemotherapy or comorbidities with steatohepatitis were observed. Most patients (74%) had no fibrosis. PCI was observed in all the patients, and more than sporadic PCI was observed in 51%. A similar proportion of more than sporadic PCI was observed in patients with steatohepatitis (57%).

DQS for the whole cohort of patients estimated 2.9% steatosis in median (1.2–6.8%). In the group with marked steatosis DQS showed 10.8% (10.1–15.3%) steatosis compared to 2.1% (1.0–3.4%) in the group without ($p<0.01$). Significant difference in DQS was also found in the group with steatohepatitis, 10.6% (10.4–17.9%) compared to the group without, 2.3% (1.0–3.7%) ($p<0.01$). No difference was seen in DQS in the group with more than a sporadic PCI 3.6% (1.9–8.6%) compared to 2.9% (1.0–7.5%) in the group without ($p>0.05$).

Steatosis assessed by ^{1}H MRS was 5.0% in median (2.9–11.0%) in the whole group of patients. In the group of patients with marked steatosis the ^{1}H MRS assessed 14.0% (12.1–15.9%) of steatosis, which was significantly higher than in the group without 3.3% (2.0–6.5%) ($p<0.01$). ^{1}H MRS measured steatosis in the group with steatohepatitis was significantly higher com-

pared to the group without steatohepatitis, 14.3% (13.2–16.7%) and 3.6% (2.0–7.6%), respectively ($p<0.001$). There was no difference seen between the groups with more than a sporadic PCI compared to the group with sporadic or no PCI 4.0% (1.9–11.7%) vs. 6.2% (3.2–11.0%), ($p>0.05$).

Comparison of DQS and ^{1}H MRS indicated a strong agreement with interclass correlation coefficient 0.955. The Bland-Altman analysis indicated that both methods consistently provided similar measures with clinically unimportant bias, 2.1%, randomly distributed differences on the whole interval of measured steatosis levels and with 95% limits of agreement of –3.1 to 7.3 %.

^{1}H MRS was the best predictor of marked steatosis, with AUC 0.983 sensitivity 100% and specificity 92%, compared to ROC curves of the weight or BMI ($p<0.05$), Figure 1a. ROC analysis also showed greater AUC of ^{1}H MRS 0.975 (sensitivity 100% and specificity 89%) than BMI in prediction of steatohepatitis defined as NAS ≥4, Figure 1b, but the difference was not statistically significant.

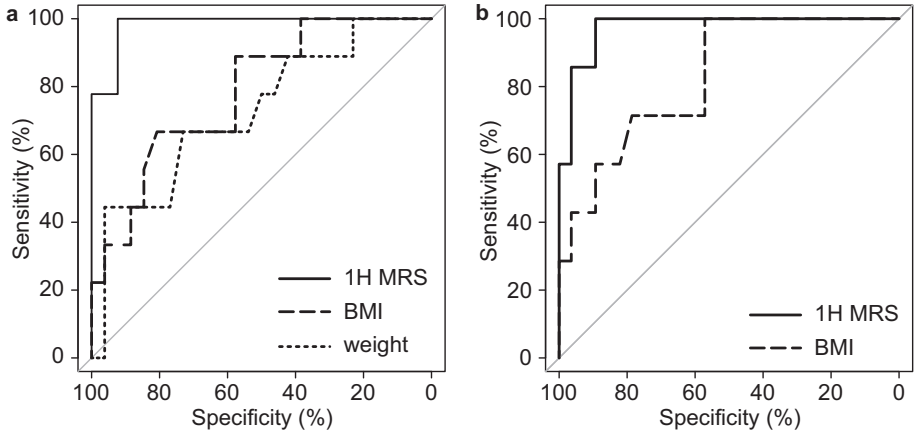

Figure 1. ROC curves for steatosis and steatohepatitis estimation. ROC analysis curves showing diagnostic accuracy of weight, BMI and ^{1}H MRS in marked steatosis prediction (a) and BMI an^{1}H MRS in steatohepatitis defined as NAS ≥4 (b).

To obtain the relative weight of each subscore in the total NAS value, the proportion of the subscore sum to the total sum of each NAS value was calculated. With increasing NAS there was no significant trend in steatosis proportion ($p=0.221$), but a decreasing trend in lobular inflammation proportion ($p=0.027$) and an increasing trend in hepatocyte ballooning proportion ($p=0.004$) was found.

5.2 Paper II

29 patients (20 male and 9 female), who fulfilled the MRIF protocol and in whom appropriate liver tissue samples were obtained, were analyzed. 72% of the patients received OX in combination with FLv or Xeloda. Histopathological analysis observed SI defined as CVI ≥3 in 21% of the patients. Median CVI score in the group of patients with SI was 5 (4–6). 69% of the patients had no fibrosis at all and the remaining patients had only mild or moderate zone 3 perisinusoidal fibrosis. All the patients with SI received OX, however Fisher exact test could not prove a significant association (p=0.148). For further comparison, three groups of patients were defined: without oxaliplatin-based chemotherapy (noOX), with oxaliplatin-based therapy without sinusoidal injury (OX+noSI) and with oxaliplatin-based therapy with sinusoidal injury (OX+SI).

Portal vein cross-section area distribution was significantly different between the groups (p=0.005). Portal vein dilatation was observed in OX+SI group (Bonferroni corrected p=0.003) and OX+noSI group (Bonferroni corrected p=0.039) compared to noOX group. However, no difference was observed between OX+SI and OX+noSI, Figure 2. Mean portal blood flow velocity showed a tendency to distribution differences (p=0.060). The OX+SI group showed a tendency to decreased mean flow velocity (Bonferroni corrected p=0.087) compared to OX+noSI group, Figure 2. No differences between OX+noSI and OX+SI vs. noOX were observed, (Bonferroni corrected p=1.000 and p=0.129, respectively). Portal blood flow and peak portal blood flow velocity showed no differences between the categories, p=0.241 and p=0.311, respectively.

The combination of mean flow velocity and cross-section area had the best predictive value for SI in ROC analysis, but the difference between variables and their combination was not significant. Mean flow velocity ≤14.35 cm/s together with cross-section area ≥1.55 cm^2 showed 100% sensitivity, 78% specificity, 50% positive predictive value and 100% negative predictive value in CVI ≥3 prediction.

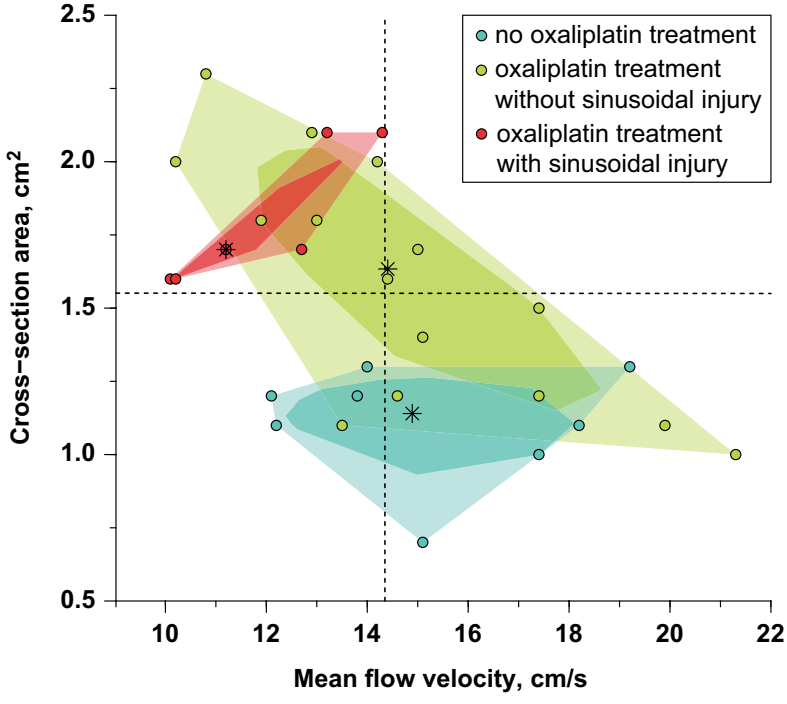

Figure 2. Bagplot for mean portal flow velocity and cross-section area. Bagplot is a bivariate generalization of the boxplot. For each of the patients groups: no oxaliplatin-based chemotherapy (blue), oxaliplatin-based chemotherapy without sinusoidal injury (green) and oxaliplatin-based chemotherapy with sinusoidal injury (red) two dimensional median (stars) is approximated, darker convex polygon, called bag, contains 50% of all points and lighter convex hull contains all the points. Actual values are showed as points on an ordinary scatter plot. Dotted lines show best performing threshold values for mean portal flow velocity (14.35 cm/s) and cross-section area (1.55 cm^2) estimated by ROC analysis.

5.3 Paper III

47 patients donated liver tissue samples to the biobank during the study period. Seven patients had no chemotherapy prior to liver surgery and represented a control group, and eight randomly selected patients (from 13 patients who exclusively received preoperative FOLFOX treatment without any biological agents) made up the treated group. There was no difference in clinical characteristics such as gender, age or BMI between the groups. Patients in the treated group received FOLFOX in a median of 5 cycles (5–6) with a 6 week (5–8) interval between FOLFOX cessation and surgery.

LC–MS/MS analysis identified 5891 proteins in the liver samples. Unsupervised hierarchical clustering by average Euclidean distance showed that

67% of technical pairs were clustered together at the first order. The treated patients were, however, mixed with controls in around 50% of the final two clusters. PCA showed a similar pattern of compact dataset with no obvious discriminating component between the study groups. The intra-individual variability was less than the inter-individual variability based on the PCA scatter plot.

The best classifying proteins between the treated group and controls were identified using an RFE–SVM feature optimization algorithm in an attempt to reach high power of enrichment analysis. The smallest number of the ranked proteins providing a minimal classification error rate of 20% was 184 (3% of all identified) proteins, Figure 3a.

The Welch t-test after manual optimization of s_0 parameter to 0.05, identified 55 (1% of all identified) proteins that were recognized as statistically significant and relevantly changed biologically between the treated and non-treated group, Figure 3b ($p < 0.05$, FDR < 0.05). Most of the identified proteins were found in the lower half of the LC–MS/MS dynamic range, i.e. expressed in low abundance. Twenty-one proteins were up-regulated with a median fold change of +2.4 (2.0–3.2) while 34 were down-regulated with a median fold change of –2.4 (3.3–2.0) in the treated group vs. controls.

The proteins included in the RFE–SVM classifying model showed a significant association with the DNA replication pathway. A greater abundance of the minichromosome maintenance (MCM) complex proteins MCM2, MCM4 and MCM7 was observed in the treated group, Table 4. This complex is involved in the process of DNA unwinding during replication. In addition, the innate immune response process was also associated with proteins of RFE-SVM model. Enrichment analysis of proteins with relevantly different abundance in the treated group compared to controls verified the role in the DNA replication pathway and process of DNA unwinding. Interaction enrichment analysis in STRING showed significantly more observed protein-protein interactions than expected by chance in significantly different and classifying protein groups ($p < 0.001$). The most confident interactions were observed between the MCM complex proteins, but proteins associated with the innate immune response interact with the whole network of identified proteins.

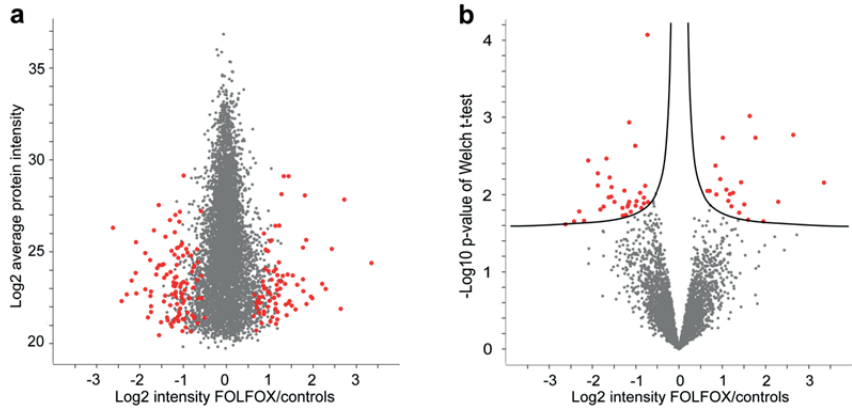

Figure 3. Classification feature optimization and Welch t-test relevantly changed proteins. Logarithm of average protein change was plotted against average protein intensity, with the 184 best classifying proteins giving a classification error rate of 20%, marked in red (a). Logarithm of average protein change is plotted against Welch t-test p-value, with relevantly changed proteins in red (b). Proteins under s_0 curves but over the Log transformed p-value threshold were statistically significant, but their biological effect was judged as marginal.

Table 4. Gene ontology and pathways. Association with significantly changed or classifying proteins.

Category	Term	Welch t-test significant proteins (n=55)			Classifying model selected proteins (n=184)		
		#	p-value FDR	Intersection genes	#	p-value FDR	Intersection genes
Biological process	DNA unwinding involved in replication	3	0.013	MCM2; MCM4; MCM7	4	0.007	MCM2; MCM4; MCM6; MCM7
	innate immune response				17	0.029	BCL2; C4B; CAMK2B; CD4; ENSG00000228284; HCK; HLA-DR4; IGKV4-1; IGLV7-43; ISG15; LGALS3; MAPKAPK3; MBL2; MX1; NCAM1; RPS6KA1; VNN1
Cellular component	MCM complex				4	<0.001	MCM4; MCM5; MCM6; MCM7
Pathway	DNA replication	3	0.013	MCM2; MCM4; MCM7	5	0.021	MCM2; MCM4; MCM5; MCM6; MCM7

5.4 Paper IV

During the study period, 471 patients (n=516 liver resections) were resected with intention to treat CRCLM. Median survival from the first liver resection was 53.7 months and 5-year survival was 46.4%. Patient characteristics, comorbidities, extent of liver metastases and operation details are summarized in Table 5. The median age of the patients was 65 years (58–71) and 62% of the patients were assessed as ASA group 2. The median BMI was 25.5 kg/m^2 (23.7–28.4) and diabetes mellitus was observed in 9% of the patients. The median Elixhauser comorbidity score (theoretical range -19 to +89) was 12 (12–16). Unilateral synchronous metastases from colon cancer were the most common indication of liver resection. The median number of metastases was two and the median of maximal tumor diameter was 25 mm. Major resection was performed in 55% of the procedures and PVE was performed 7 weeks (6–9) before 6% of the resections. The median operation time was 150 min (106–194). Median intraoperative blood loss was 1100 ml (600–2000) and erythrocytes were transfused in 45% of the operations (median 2 units (1.5–5)).

Preoperative chemotherapy for CRCLM before liver resection was introduced at our institution at the end of 1999. Nearly all of the 509 regimens included intravenous or per oral 5-fluorouracil, with the exception of second-line irinotecan monotherapy in 3% (15/509) of all the treatments. Chemotherapy was administered before resection in 75% of the procedures, within one year in 68%, within 6 months in 63% and within 30 days in 16% (Table 6). Oxaliplatin was the main compound of the first-line chemotherapy prior to 60% of the liver resections with a median of 6 cycles and a median interval of 7 weeks to resection. Irinotecan, mostly used as a second-line therapy, was administered in a median of 7 cycles in 18% of the resections with a median interval of 7 weeks to surgery. In 11% of the resections both oxaliplatin and irinotecan were used before liver resection.

Preoperative chemotherapy was mainly used in younger patients with lower ASA classes. However, preoperative chemotherapy indication was not influenced by comorbidities such as obesity, diabetes mellitus or higher Elixhauser comorbidity score. Synchronous metastatic disease and the number of metastases were mostly associated with oxaliplatin-based preoperative chemotherapy. Re-resections were associated with irinotecan-based (second-line) therapies. The number of metastases, major resections and previous PVE were associated with preoperative chemotherapy. Both major resections and PVE were associated with oxaliplatin-based (first-line) therapies. All the data are included in supplementary material to paper IV.

Table 5. Clinical characteristics of resections (n=516).

		n median	(%) [interquartile range]
PATIENT			
Gender	Male	324	(63)
Age	≥70 years	147	(28)
ASA	1	88	(17)
	2	320	(62)
	3	108	(21)
BMI	<25 kg/m^2	222	(43)
	≥25 and <30 kg/m^2	212	(41)
	≥30 kg/m^2	82	(16)
Diabetes mellitus		48	(9)
Elixhauser comorbidity score	≥13	214	(41)
DISEASE			
Primary tumor site	colon	294	(57)
	rectum	214	(41)
	both	8	(2)
Synchronous metastases		289	(56)
Bilobar metastases		207	(40)
Number of metastases (#)		2	[1–3]
Maximal tumor diameter (mm)		25	[20–40]
OPERATION			
Re-resection		47	(9)
Portal vein embolization		32	(6)
Major resection		281	(54)
Vascular system procedure		2	(0)
Biliary system procedure		3	(1)
Resection with primary tumor operation		18	(3)
Operation time (min)	≥150 min	256	(50)
Blood loss (ml)	≥1100 ml	260	(50)
Any transfusion of erythrocytes intraoperative or directly postoperative		233	(45)

Table 6. Characteristics of preoperative chemotherapy stratified by comorbidities. Total number proportion is counted from number of resections (n=516). Proportions in comorbidity categories are counted from respective total number in each row.

	Total n (%)		Age ≥70 years		BMI <25 kg/m^2		BMI ≥25 and <30 kg/m^2		BMI ≥30 kg/m^2		Diabetes mellitus		Elixhauser comorbidity score ≥13	
Chemotherapy	389	(75)	94	(24)	161	(41)	167	(43)	61	(16)	37	(10)	155	(40)
Number of chemotherapy cycles >4	315	(61)	75	(24)	125	(40)	141	(45)	49	(16)	31	(10)	126	(40)
Never	127	(25)	53	(42)	61	(48)	45	(35)	21	(17)	11	(9)	59	(47)
More than 1 year before resection	39	(8)	9	(23)	15	(39)	18	(46)	6	(15)	3	(8)	14	(36)
Between 1 year and 6 months before resection	26	(5)	5	(19)	15	(58)	7	(27)	4	(15)	1	(4)	11	(42)
Between 6 months and 30 days before resection	242	(47)	66	(27)	106	(44)	96	(40)	40	(17)	27	(11)	93	(39)
Under 30 days before resection	82	(16)	14	(17)	25	(31)	46	(56)	11	(13)	6	(7)	37	(45)
Oxaliplatin	309	(60)	72	(23)	135	(44)	127	(41)	47	(15)	25	(8)	121	(39)
Number of oxaliplatin cycles >4	218	(42)	47	(22)	91	(42)	94	(43)	33	(15)	19	(9)	86	(39)
Irinotecan	95	(18)	17	(18)	30	(32)	49	(52)	16	(17)	7	(7)	29	(31)
Number of irinotecan cycles >4	70	(14)	13	(19)	21	(30)	38	(54)	11	(16)	6	(9)	20	(29)

Short-term resection outcomes are presented in Table 7. 90-day mortality (1.9%) was associated with complications from 0.6% if no liver-specific complication occurred up to 6.2% if all three complications were combined. 90-day mortality was associated with grade C of all three liver-specific complications and increased in frequency up to 50% for PHH.

Post-hepatectomy hemorrhage

PHH was observed in 19% of the resections, most of which were grade A. Univariate analysis demonstrated an association between extensive surgery (perioperative blood loss and erythrocyte transfusion (both with $p<0.001$), major resections ($p=0.001$), PVE ($p=0.031$), maximal tumor diameter ($p=0.036$), operation time over 150 minutes ($p=0.037$)) and PHH. More than four oxaliplatin cycles ($p=0.016$) and termination of oxaliplatin within 30 days before surgery ($p=0.031$) were also associated with PHH. Termination of chemotherapy treatment more than 1 year before surgery was protective against PHH in univariate analysis.

Multivariate logistic regression identified independent predictors of outcomes (Table 8). PHH was associated with more than four oxaliplatin cycles (RR 1.4, $p=0.047$), blood loss over 1100 ml and erythrocyte transfusion and synchronous metastases without any significantly modulating factors.

Biliary leakage

BL was observed in 12% of the patients, mainly grade B. In univariate analysis BL was associated with biliary system procedures ($p=0.026$), major resections ($p<0.001$), more than four oxaliplatin cycles ($p=0.018$), blood loss over 1100 ml and operation time over 150 minutes ($p=0.003$).

BL was only independently associated with the biliary system procedures and major resections (Table 8).

Post-hepatectomy liver failure

PHLF was observed in 26% of the resections, mostly grade B and A, see Table 7 for details. Most of the univariate predictors were associated with PHLF. Extensive surgery: major resection ($p<0.001$), PVE ($p=0.021$), number of metastases ($p=0.005$), maximal tumor diameter ($p=0.010$), blood loss over 1100 ml, erythrocyte transfusion and operation time over 150 minutes (all with $p<0.001$) increased the risk for PHLF. Concerning the time aspect of preoperative chemotherapy ($p=0.066$, 0.033), and particularly OX ($p=0.004$, 0.010), cessation 6 months respective 30 days before surgery was associated with PHLF. More than four chemotherapy cycles ($p=0.037$) and in particular more than four OX cycles ($p=0.002$) were other univariate risk factors. Moreover, male gender ($p=0.008$) and synchronous metastases ($p=0.027$) were risk factors. Re-resections ($p=0.009$) and resections performed in the recent half of the study period ($p=0.017$) were associated with

lower risk of PHLF. 87% of the re-resections were performed as minor re-sections.

Table 7. Short-term resection outcomes. Relation to liver specific complications grading (n=516).

OUTCOMES	All patients (n=516)		90-day mortality for respective category		Chi-square p-value	Bonferroni corrected p-value
	n	(%)	n	(%)		
30-day mortality	3	(0.6)				
90-day mortality	10	(1.9)				
Post-hepatectomy haemorrhage						
No	416	(80.6)	7	(1.7)	0.416	
Yes	100	(19.4)	3	(3.0)		
grade A	83	(16.1)	1	(1.2)		
grade B	13	(2.5)	0	(0.0)	<0.001	
grade C	4	(0.8)	2	(50.0)		<0.05
Bile leakage						
No	456	(88.4)	9	(2.0)	1.000	
Yes	60	(11.6)	1	(1.7)		
grade A	3	(0.6)	0	(0.0)		
grade B	52	(10.1)	0	(0.0)	0.022	
grade C	5	(1.0)	1	(20.0)		<0.05
Post-hepatectomy liver failure						
No	382	(74.0)	2	(0.5)	<0.001	<0.05
Yes	134	(26.0)	8	(6.0)		<0.05
grade A	43	(8.3)	2	(4.7)		
grade B	77	(14.9)	2	(2.6)	<0.001	
grade C	14	(2.7)	4	(28.6)		<0.05
Liver-specific complications prevalence						
None	309	(59.9)	2	(0.6)		
Only one	136	(26.4)	5	(3.7)	0.064	
Combination of two	55	(10.7)	2	(3.6)		
Combination of all three	16	(3.1)	1	(6.3)		
Dindo-Clavien complication						
<III.b	455	(88.2)	2	(0.4)	<0.001	<0.05
≥III.b	61	(11.8)	8	(13.1)		<0.05

PHLF was independently associated with major resections, perioperative erythrocyte transfusion and male gender. Rectal cancer metastases proved to be an independently protective effect on PHLF, see Table 8.

Severe complications

Severe complications (Dindo-Clavien grade ≥IIIb) were observed in 12% of the resections, requiring treatment in general anesthesia or intensive care. Patients were discharged directly to home after 87% of the resections at median postoperative day 9 (8–12). Severe complications were associated with a combination of several of the above-mentioned factors describing the extent of surgery ($p < 0.001$–0.019) and operation outcomes ($p < 0.001$–0.043) according to univariate analysis.

Multivariate analysis identified major resections, perioperative blood loss over 1100 ml and number of metastases as independently associated with severe complications (Table 8). Age over 70 years was the only modulating factor recognized in the study, which increased risk for severe complications (RR 1.7, $p=0.033$).

90-day mortality

90-day mortality, due to partial overlap with the Dindo-Clavien score grade V, showed a similar association with characteristics of metastases. In addition, patient comorbidities (underlying liver disease ($p=0.003$) and diabetes mellitus ($p=0.003$)), showed an important association.

Finally, risk for 90-day mortality was independently higher with underlying liver disease (RR 8.7, $p=0.015$) and diabetes mellitus (RR 6.4, $p=0.007$), number of metastases and maximal diameter (Table 8).

Table 8. Multivariate logistic regression. Patient, disease, operation and chemotherapy characteristics associated with short-term outcomes.

	total	PHH		BL		PHLF		Severe Clavien		90-day mortality	
	n (%)	RR (95% CI)	p-value	RR (95% CI)	p-value	RR (95% CI)	p-value	RR (95% CI)	p-value	RR (95% CI)	p-value
PATIENT											
Gender - male	324 (63)					1.7 (1.2-2.2)	0.003				
Age >70 years	147 (28)							1.7 (1.0-2.7)	0.033		
Liver disease	12 (2)									8.7 (1.6-29.4)	0.015
Diabetes mellitus	48 (9)									6.4 (1.7-19.1)	0.007
DISEASE											
Primary tumor - rectum	214 (41)					0.5 (0.3-0.8)	0.001				
Synchronous metastases	289 (56)	1.5 (1.0-2.0)	0.038								
Number of metas-tases								1.1 (1.0-1.2)	0.021	1.2 (1.0-1.3)	0.032
Maximal tumor diameter										1.0 (1.0-1.0)	0.002
CHEMOTHERAPY											
Oxaliplatin cycles >4	218 (42)	1.4 (1.0-2.0)	0.047								
OPERATION											
Major resection	281 (54)			4.3 (2.7-6.0)	<0.001	3.6 (3.3-3.8)	<0.001	3.1 (1.8-4.7)	<0.001		
Biliary Procedure	3 (1)			6.2 (1.3-8.4)	0.031						
Blood loss >1100ml	260 (50)	2.0 (1.4-2.8)	0.001					2.0 (1.2-3.1)	0.011		
Any intraoperative erythrocytes transfusion	233 (45)	1.9 (1.3-2.6)	0.002			2.0 (1.5-2.4)	<0.001				

6 Discussion

Papers I and II were focused on the non-invasive diagnosis of non-tumorous liver parenchyma changes known to be associated with preoperative chemotherapy for CRCLM. The histopathological diagnoses of CASH and SI seem to be two different, non-overlapping entities, as demonstrated in Figure 4, similar to the work of Brouquet et al. (45). This claim is supported by their different underlying mechanisms. On the contrary, in most reports it is not possible to deduce if these diagnoses occur together in the same individual patient (27-29, 104, 105).

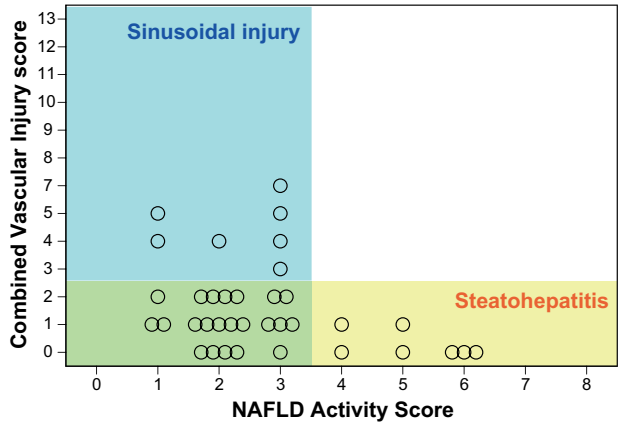

Figure 4. NAS and CVI scores of patients included in papers I and II. NAS ≥4 defines steatohepatitis and CVI score ≥3 defines sinusoidal injury (SI). Dot plot demonstrates two different non-overlapping histopathological diagnoses known to be associated with preoperative chemotherapy for CRCLM (unpublished data).

6.1 Discussion paper I

Three different methods of assessing liver steatosis and steatohepatitis were compared: ^{1}H MRS, DQS and histopathology, using specimens from patients who had undergone resection for CRCLM. ^{1}H MRS could predict with high sensitivity and specificity not only marked steatosis (100% and 92%), but also steatohepatitis as defined by the NAS (100% and 89%), without refer-

ence to the other components of the NAS score (lobular inflammation and ballooning). NAFLD activity increases steadily with precisely measured steatosis levels. Our data could not demonstrate an association between preoperative chemotherapy and marked steatosis or steatohepatitis. Interestingly, 5 of 6 patients treated with IR showed no marked steatosis or steatohepatitis, similar to the findings of Ryan et al (16). The specificity of body weight and BMI as predictors of steatosis or steatohepatitis was very low, 23–57%.

MR spectroscopic imaging demonstrated good diagnostic precision for steatosis and steatohepatitis, but was unable to discriminate between them (56). Nor was ^{1}H MRS in paper I able to do so. However, we do not believe that the ability to discriminate in this way provides any clinical benefit. NAS was developed for complex NAFLD evaluation, where steatohepatitis is just a part of the full disease spectrum. We used separated steatosis categories solely for methodological purposes, making it possible to carry out reliability analyses of steatosis assessment methods. Paper I showed a higher sensitivity but a similar specificity of ^{1}H MRS in steatosis prediction to those found by van Werven et al (54). The difference may be explained by a smaller cut-off value used by van Werven et al for the histopathological definition of steatosis; 5% vs 33%, respectively. Steatosis levels measured by ^{1}H MRS in paper I were relatively low (median 5%). It should be noted that the sensitivity of ^{1}H MRS at these levels is higher than that of standard MR imaging methods (106). Our patients presented with none or just mild zone 3 perisinusoidal fibrosis, which should not influence the measurement of steatosis levels, as it did in the study by McPherson et al (107).

The gold standard of liver steatosis assessment, histopathology of liver biopsies, has been questioned by El-Badry et al (51). Assessment of steatosis was strongly observer-dependent, not reproducible due to possible sampling error, and did not correlate well with DQS, as confirmed by other groups (108). DQS showed good correlation with ^{1}H MRS in patients with NAFLD (109). Histopathological assessment showed higher levels of steatosis, but lower reliability when compared to ^{1}H MRS or DQS. This could be explained by the fact that histopathological examination assesses the total area of steatotic hepatocytes and not exclusively the area of fat droplets as measured by DQS. The concordance we found between ^{1}H MRS and DQS can be explained by the fact that we used relatively large volumes of resected liver parenchyma for histopathology, just 2.4× smaller than ^{1}H MRS volume of interest and 400× larger than standard biopsies.

Another interesting finding in paper I was the high level of PCI, a marker of advanced NAFLD, together with a relatively low expression of active steatohepatitis (NAS). This has not been reported before and our data offer no easy explanation. The proportion of more than sporadic PCI was the same in patients with and without steatohepatitis, and steatosis levels measured by ^{1}H MRS were similar. It could perhaps be regarded as a sign of healed liver parenchyma following chemotherapy injury, similar to the effect of weight

reduction after bariatric procedures on the liver of obese patients with NAFLD (110).

One limitation of paper I is that the DQS method has yet to be standardized as regards the applied algorithm for determination of the image intensity threshold and other steps in the analysis. Despite the exclusion of microsteatosis (droplets diameter <2.2 μm), a comparison of DQS and ^{1}H MRS on a Bland-Altman plot demonstrated no consistent underestimation in patients with low steatosis levels (<5%), in whom this type of error would have been most obvious. No patients with the highest NAS (7 and 8 points) were included in the study. These patients usually have severe and clinically apparent steatohepatitis, which may exclude them from liver resection. Relatively large volume of interest (27 cm^3) should prevent any possible lipid distribution inhomogeneity affecting results (55, 111). Paper I lacks a validated reference standards for liver fat content quantification, however, Roldan-Valadez et al (112) showed a strong correlation between microcolorimetrically assessed lipid content in liver samples and ^{1}H MRS.

6.2 Discussion paper II

To our knowledge, paper II was the first investigation that has evaluated changes in portal vein hemodynamics associated with SI and OX treatment using a non-invasive method. OX was associated with portal vein dilatation. In the case of established SI (defined as CVI ≥3) decreased mean portal vein flow velocity and portal vein dilatation without changes in portal vein flow were observed. Such changes in portal vein hemodynamics could be interpreted as indirect signs of greater liver parenchyma resistance to blood flow in the sinusoids, balanced in a "new" steady state with blood congestion in the portal system, while keeping the same liver perfusion. Increased liver parenchyma resistance is usually found in cases of high-grade fibrosis and cirrhosis (64), not observed in our data, supporting the role of SI in observed changes. Others have reported an increase in spleen size after OX (61) as a consequence of SI (57), which could also be seen as an indirect sign of congestion in the portal system, Figure 5. This reversible, oxaliplatin dose dependent, increase in splenic size has also been suggested as one of the reasons for associated thrombocytopenia (57, 61), which directly influences APRI, another predictor of SI (58, 60). Cases of clinically manifest symptoms (62, 113) and liver vein wedge-catheter verified portal hypertension (63) are reported after OX induced SI. Our MRIF findings are consistent with the proposed model of SI effects following OX (62), Figure 5.

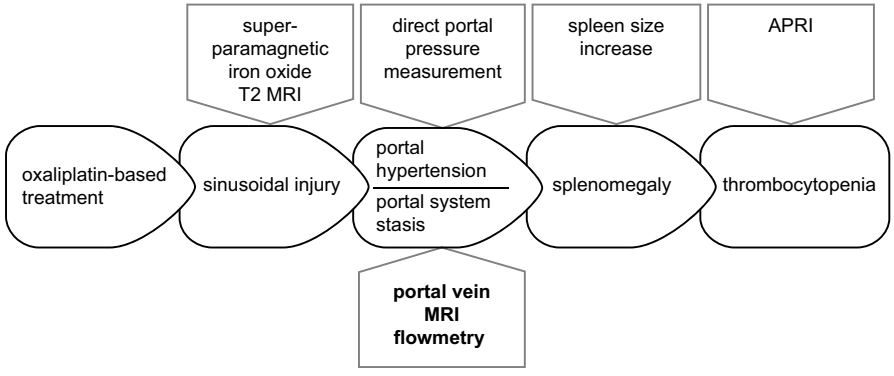

Figure 5. Theoretical model of sinusoidal injury effects caused by oxaliplatin-based treatment with the relevant methods of sinusoidal injury prediction.

In contrast to other studies, in paper II we could not demonstrate a direct association between OX and SI (16, 18), probably due to the small sample size. This was not, however, the aim of the study. All the patients with SI had received OX as was the case in a large histopathological study by Rubbia-Brandt et al (18). The period between OX cessation and liver surgery was, however, relatively long, 6 weeks (4–34). It remains unclear how long SI effect persists after chemotherapy treatment has ceased, but it is probably of a much longer duration than the commonly reported period of 5–6 weeks after preoperative treatment, Figure 6. Ryan et al observed SI in 11% of patients who had not had preoperative chemotherapy within a year before liver surgery, but prior chemotherapy treatment was not documented (16). Increase in spleen size ≥50% strongly correlates with SI and remains 6 months after OX cessation (57).

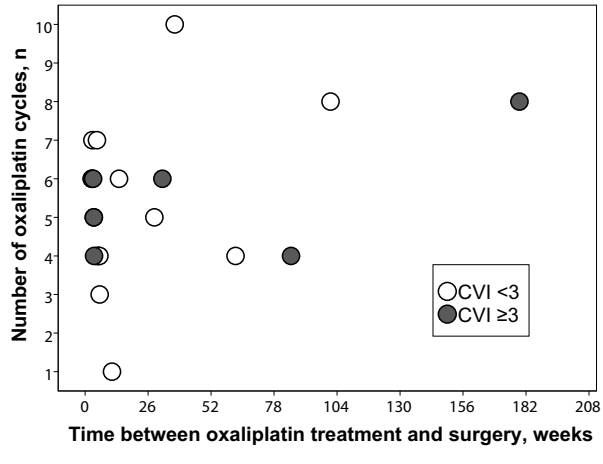

Figure 6. Scatter plot of interval between OX cessation and liver surgery versus number of OX cycles. Patients with SI (CVI ≥3) in grey and without SI (CVI <3) in white.

6.3 Discussion paper III

Paper III attempted to elucidate the underling molecular effects of OX on non-tumorous liver parenchyma. The study demonstrated differences in about 1% of identified proteins in liver parenchyma proteome 6 weeks after FOLFOX treatment cessation. Changes were associated with upregulation of MCM complex, which, via the process of DNA unwinding, increases DNA replication and indicates cell cycle entry. In addition, abundance changes were observed in innate immune response proteins. This is the first study, to our knowledge, that has analyzed the effects of FOLFOX treatment on non-tumorous human liver tissue at protein level.

The shotgun proteomics has several strengths (114). Its global character helps to minimize confirmation bias, as with microarray techniques. Isolation and preparation of more stable proteins for LC-MS/MS, compared to the limited quality and quantity of isolated RNA if complex tissue is analyzed, is an advantage of the method. Protein quantification provides insight into the results of the gene expression chain and also reflects posttranscriptional regulation (115), whilst the quantity of mRNA copies does not necessarily reflect the quantity of translated protein (116).

Protein expressions in the study groups were similar. Unsupervised hierarchical cluster analysis, PCA and RFE-SVM classification showed no high discriminating components.

Acute hepatocyte injury in cultured rat hepatocytes after exposure to cisplatin (platinum-based cytostatic similar to oxaliplatin) for 24 hours revealed significant changes in 29% (95/325) of quantified proteins (117), in contrast to 1% in our current data. Hepatocytes occupy almost 80% and all the other cell populations only 6.5% of total liver volume (118), suggesting that the observed proteome changes mainly reflected changes in hepatocytes. This leads to the explanation, that recovery from acute FOLFOX injury during the 6 weeks may decrease the effects on the proteome, as may be seen in the reversion of clinical signs of liver injury during the same time (57). In addition, this discrepancy may be partially explained by the variability of the whole liver tissue proteome, which can conceal changes observable in separated cell populations (117).

The main mechanism of action of oxaliplatin is the formation of platinum-DNA adducts leading, through retarded replication and transcription, to apoptotic cell death (119). Although the mitosis and apoptosis rate in liver is normally so low (<0.1%) that it is difficult to estimate (120); increased DNA replication (121) and MCM complex expression (122) were observed in the treated group. Increased expression of MCM2 is a sensitive marker of cell cycle entry (123). The observed changes in the treated group may represent a compensatory effect/recovery of non-tumorous liver tissue from the effects of FOLFOX (124). The association of FOLFOX treatment with changes in the expression of proteins involved in the innate immune response is more

complex. The identified proteins are associated with processes of oxidative-stress response and ischemia-induced cell death (CAMK2B, VNN1), apoptosis (BCL2, LGALS3), complement activation (C4B, MBL2) and extracellular matrix remodelation (VCAN, NCAM1), but none were verified in enrichment analysis. This may implicate that the initial signaling is attenuated and does not proceed to further changes in proteome, or that the liver has already recovered from the effects of FOLFOX and only residual proteome changes were observed. Cell cycle entry may also reflect the onset of nodular regenerative hyperplasia, one of histopathological patterns in SI (18). Associated changes may be masked by OX+noSI patients being in the same (treated) group.

Identified proteome changes were mostly in the lower part of the LC-MS/MS dynamic range, Figure 3a, which may be influenced by missing data imputation. In the paper III no histopathological diagnosis of SI was made, which makes any comparison with the studies focused on SI-associated transcriptome changes problematic. Pathways recognized to be important for SI development in microarray study of Rubbia-Brandt et al were not overlapping with our proteome analysis, although there was an overlap of 46% of recognized proteins from the original set of 913 genes. This is probably due to the use of both patients with and without FOLFOX treatment, in the control group (without SI) in the microarray analysis (34). Proteins associated with the SI mechanisms mentioned in the introduction were either not changed or not detected in the present study. Nevertheless, the classifying proteins demonstrated a small, but significant (Fisher exact test $p=0.047$) overlap with genes associated with SI in comparison with the microarray study by Agostini et al (30). The overlap of COL3A1, VCAN and TMPRSS6, was significant although only one third (26/81) of the original list of genes was identified in the present paper. These proteins are important in extracellular matrix remodelation, but no association with such enrichment category was observed.

6.4 Discussion paper IV

Paper IV demonstrates the safety of liver resection in combination with modern preoperative chemotherapy for CRCLM in a large retrospective, single-center cohort composed of consecutive liver resections. Outcomes in the meaning of 30- and 90-day mortality and severe complications are comparable with what has been reported from large-volume referral centers (74). Patient characteristics (e.g., male gender, aged over 70, and comorbidities such as underlying liver disease and diabetes mellitus) were demonstrated to have a negative impact on short-term outcomes. Higher dose of oxaliplatin was associated with a clinically important risk for PHH, especially for those patients with initially resectable metastases. Moreover, the association be-

tween the procedure characteristics (such as major resection, intraoperative blood loss and erythrocyte transfusions (66) with liver-specific complications) was verified in the present study. To our knowledge, there is no detailed report on postoperative liver-specific complications after resections for CRCLM as defined according to the ISGLS.

The association between preoperative chemotherapy and operation time and blood loss can be explained by their association with tumor burden and the need for more extensive surgery (data not shown). Our data could not provide any evidence of the impact of the time interval between last chemotherapy treatment and resection on short-term outcomes. From chemotherapy details, only more than four oxaliplatin cycles showed an association with any of the studied outcomes.

During the study period, the use of chemotherapy has changed in several characteristics. Most importantly, preoperative chemotherapy was used more often in the recent period (68% vs. 83%, $p<0.001$). Also the proportion of patients treated within 6 months increased from 22% to 45%, but patients treated within 30 days before resection decreased from 16% to 9% (both with Bonferroni corrected $p<0.050$). In a subgroup of OX treated patients median number of OX cycles went from 6 to 5 ($p <0.001$) and in a subgroup of IR treated patients the median number of IR cycles from 9 to 5 ($p=0.002$), respectively. This change was mainly due to the intention to reduce the preoperative doses in primarily resectable cases to reduce the presumed risk of high-dose-associated toxicity. A relatively uniform (median 6 weeks) long period between cessation of chemotherapy and liver surgery during the entire study period is probably the reason why our data could not verify the hypothesis that the time interval alone is a risk factor for complications (87).

The Elixhauser comorbidity score (98) was selected as a measure of comorbidity in patients, whereas Elixhauser's original algorithm showed high accuracy in predicting in-hospital mortality after liver resection (125) and weighted comorbidity assessment systems have been advocated as more accurate for short-term prognosis (126).

The retrospective design of the present study, although based on a prospectively accumulated data register, may result in some limitations to be considered. While all consecutive resections were studied and an analysis was done aimed to evaluate the association between preoperative characteristics and short-term resection outcomes only, unreported dropout under chemotherapy treatment before planned surgery is not an important limitation of this study. On the other hand, an absence of routine pathological evaluation of non-tumorous liver parenchyma for patterns of chemotherapy toxicity may be seen as a major limitation of the study. However, this may be difficult to achieve due to availability of only small amounts of non-tumorous liver parenchyma, which also may be damaged by surgical trauma during limited non-anatomic resections, or influenced by remodeling of microarchitecture by the metastases themselves (44).

7 Conclusions

- ^{1}H MRS was demonstrated to be a reliable non-invasive tool for the evaluation of steatosis and possibly steatohepatitis as defined by NAS criteria. ^{1}H MRS concurred with the liver steatosis estimation by DQS.

- Oxaliplatin treatment was associated with portal vein dilatation estimated by MRIF, and patients with SI showed a tendency towards decreased mean portal flow velocity. Portal vein hemodynamics variables estimated by MRIF can identify patients without SI non-invasively.

- A small proportion (1%) of identified non-tumorous human liver tissue proteome was found to be changed in patients 6 weeks after FOLFOX treatment. Changes were associated with cell cycle entry through MCM complex activity and with the innate immune response. The hypothesis was put forward that only residual proteome changes were observed and that the liver had already recovered from the effects of FOLFOX treatment.

- The size of resection, intraoperative blood loss and erythrocyte transfusion were associated with liver-specific and general complications after surgery for CRCLM. Treatment with more than four OX cycles increases the risk of PHH. Comorbidities such as underlying liver disease and diabetes mellitus were risk factors affecting 90-day mortality.

8 Future perspectives

Non-invasive MR methods for monitoring the effects of chemotherapy on non-tumorous liver parenchyma may help to identify patients at risk of complications associated with liver resection for CRCLM. It can help to individualize preoperative chemotherapy by optimizing the number of cycles and the timing of liver surgery after chemotherapy, with the aim of reducing the risk of postoperative complications. ^{1}H MRS showed good reliability, but requires special coils not available in standard clinical MR equipment. The application of Dixon's phase-contrast imaging method offers a way of extending NAS prediction into routine clinical practice. Finding a specific non-invasive tool for identifying SI changes during preoperative chemotherapy represents a further step forward in the monitoring of the effects of chemotherapy. In an hitherto unpublished manuscript, not included in this thesis, we have identified that APRI in combination with mean portal flow velocity were highly sensitive and specific predictors of SI. MRIF surveillance of portal vein hemodynamic changes is needed before, during and after OX. This may help to identify the onset of SI.

The incidence of severe liver-specific complications affecting 90-day mortality is low. Nevertheless, patients identified by non-invasive monitoring to be at high risk may benefit from intervention prior to liver resection. A low calorie diet for a few weeks before resection may, for example, reduce the grade of steatosis and possibly reduce the risk of complications. Similarly, identifying patients at risk of SI may provide an indication for adding bevacizumab to the first-line OX. This combination has been shown to have the capacity to protect liver parenchyma from SI during OX treatment.

Further research into the effects of oxaliplatin on non-tumorous cells may reveal why drug concentrations vary so much despite the use of identical doses per m^2 of body surface. Assuming that adverse effects are dose dependent, the identification of patients at risk may help to further individualize preoperative chemotherapy and improve the future results of the multimodal treatment of CRCLM.

9 Summary of the thesis in Swedish

Populärvetenskaplig sammanfattning

Modern behandling av levermetastaser från ändtarms- och tjocktarmscancer (CRCLM) är ofta en kombination av kemoterapi och kirurgi. Pre- och post operativ kemoterapi och leverresektion ger en 5-års överlevnad i storleksordning 40–50%. Preoperativ kemoterapi bedöms kunna ha effekt på mikrometastatisk sjukdom. Med sådan behandling kan primärt icke resektabla tumörer göras resektabla. Den bedömning av behandlingsresultatet som görs ger samtidigt en bild av tumörsjukdomens biologiska aktivitet. Detta tillsammans har givit dokumenterad förlängd sjukdomsfri överlevnad.

Preoperativ kemoterapi har uppenbara fördelar men också flera potentiella nackdelar, vilket diskuterats på senare tid. Fr a har den diskussionen fokuserats på risken för kemoterapi associerad toxisk effekt på icke tumörbärande leverparenkym. Leverförfettning, steatos, har observerats på patienter som behandlas med fluorouracil-leucovorin, som oftast ingår i modern kemoterapi mot CRCLM. Iroinotecan har associerats med steatohepatit (CASH) och oxaliplation med sinusoidskada (SI). Såväl CASH som SI kan förekomma utan att ge några kliniska manifestationer under eller efter kemoterapi. I flera studier har man försökt hitta faktorer som förutsäger CASH eller SI innan leverkirurgi, men ännu finns ingen pålitlig icke-invasiv metod för detta. Preoperativ leverbiopsi, en invasiv teknik, rekommenderas inte då den erhållna ringa mängden levervävnad ger risk för felaktiga slutsatser.

Rutinundersökning med MR, datortomografi eller ultraljud, ger ingen säker bild av graden av steatos och kan inte detektera CASH. Proton MR spectroscopi (^{1}H MRS) anses vara den känsligaste icke-invasiva metoden att detektera steatos. I **arbete I** gjordes försök att värdera graden av steatos före leverresektion. ^{1}H MRS dagen före operation jämfördes med rutinmässig histopatologisk värdering av operationspreparatet avseende grad av steatos, steatohepatit och med histopatologisk digital kvantifiering (DQS) av steatos, som uppfattas vara den pålitligaste metoden. Rutinmässig histopatologisk värdering visades vara mindre pålitlig som metod jämfört med ^{1}H MRS och DQS. De två sistnämnda visade hög grad av samstämmighet. ^{1}H MRS dagen före operation kunde förutsäga CASH med 100 % sensitivitet och 89 % specificitet utan att ta hänsyn till lobulär inflammation eller hepatocytsvullnad, som utgör andra tecken på CASH.

Metoder som bestämning av kvoten mellan mängden av enzymet aspartat aminotransferas och antalet trombocyter i blod (APRI), av mjältens ökade storlek under eller efter behandling och superparamagnetisk järnoxid inriktad MRI kan användes för att förutsäga SI inför leverkirurgi. De första metoderna baseras på hypotesen att SI orsakar portal hypertension, vilket följs av ökad mjältstorlek och minskat antal trombocyter i blodet. I **arbete II** studerades hemodynamiska förändringar i vena portae utnyttjande MR flödesmätningsteknik (MRIF) på patienter som behandlats med oxaliplatin och som utvecklat eller inte utvecklat SI. Oxaliplatinbehandling visades vara associerad med portal dilatation. Patienter med SI hade tendens till minskad flödeshastighet i portavenen, men mängden blodflöde var inte förändrad. Detta kan uppfattas tyda på att SI leder till ett ökat blodflödesmotstånd i leverparenkymet och stas i portavensystemet. Genom att mäta hemodynamiska variabler i vena portae med MRIF kunde patienter utan SI identifieras med 100 % sensitivitet och 78 % specificitet.

Den exakta mekanismen med vilken oxaliplatin påverkar det icke tumörbärande leverparenkymet är okänd. Studier utnyttjande microarray teknik, som försökt värdera hela panoramat av oxaliplatin-orsakade förändringar, har visat påverkan av angiogenes, cellulära adhesionsmekanismer och förändringar i extracellulära matrix men också aktivering av akut fas respons, av koagulationssystemet, hypoxiska faktorer och fibrosutveckling i levern. Biokemisk aktivering som leder till förändringar i den sinusoidala arkitekturen i levern och till obstruktion av blodflödet där beskrevs i arbete II. Senare utvecklas skadan till förlust av hepatocyter, mild fibros och som slutstadium nodulär regenerativ hyperplasi. I **arbete III** gjordes ett försök att visa de effekter en oxiliplatinbaserad behandling (FOLFOX) har på leverparenkymet genom att kvantifiera uttryck av proteiner med användning av en global proteomanalys. Icke tumörbärande levervävnad från 15 patienter studerades. Totalt identifierades 5891 proteiner varav maskinellt 3% uppfattades som klassificerande proteiner. Dessa var associerade med förändringar i DNA replikationen och med immunförsvaret. Signifikanta förändringar i mängd observerades 6 veckor efter oxilaplatinbehandlingens slut i 1 % av proteinerna. Förändringarna i DNA replikation mekanismen stödjer att oxaliplatinbehandlingen har påverkan på cellcykeln. Resultaten stödjer hypotesen att levern till viss del redan regenererat efter FOLFOX behandlingen 6 veckor efter behandlingens slut.

Den kliniska relevansen av de studerade förändringarna i icke tumörbärande leverparenkym har varit föremål för flera studier. Initialt rapporterades alarmerande hög 90 dagars mortalitet bland patienter med CASH. Kraftig SI rapporterades associerad med ökad risk för blödning under och efter leverkirurgi och ökad postoperativ morbiditet. I andra studier, som hävdades vara baserade på mer korrekt sammansatta patientmaterial, ifrågasattes den kliniska relevansen av de resultaten. Faktorer som sjukdomsbördan, leverresektionens nödvändiga omfattning i syfte att nå tumörfrihet och blodförlus-

tens storlek vid kirurgin är kända riskfaktorer för postoperativa komplikationer. Å andra sidan är effekten av patientrelaterade faktorer som andra samtidiga allvarliga sjukdomar inte klarlagd. Man vet att steatos orsakad av fetma eller diabetes utgör riskfaktorer. Ingen enskild riskfaktor, förenad med ökad risk för komplikationer vid leverkirurgi, har identifierats utan korttidsresultatet efter leverkirurgi för CRCLM tycks vara associerat med kombinationer av olika faktorer. **Arbete IV** försökte identifiera olika tänkbara karakteristika gällande patient, sjukdom och kemoterapi och deras samband med leverspecifika postoperativa komplikationer definierade enligt Internatinal Study Group of Liver Surgery. Blödning (PHH), läckage av galla (BL), sviktande leverfunktion (PHLF) och andra allvarliga komplikationer efter leverkirurgi identifierades och graderades in en retrospektiv analys av ett prospektivt samlat material från ett leverkirurgiskt centrum. Totalt studerades 516 konsekutiva leverresektioner för CRCLM på 471 patienter. Studien verifierade att resektionens omfattning, blodförlustens storlek och transfusionsbehovet var av vikt för utvecklandet av leverspecifika postoperativa komplikationer. Kemoterapi med mer än 4 cykler oxaliplatin hade samband med PHH, men inte med andra typer av komplikationer. Förekom det flera leverspecifika komplikationer postoperativt ökade risken för död inom 90 dagar.

Med hänsyn till dessa data föreslås att preoperativ kemoterapi balanseras försiktigt mot kända riskfaktorer i syfte att ytterligare förbättra det postoperativa resultatet efter leverkirurgi vid CRCLM.

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