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Liver regeneration and function following portal vein occlusion and hepatectomy

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‘Aluta Continua, Victoria Acerta’

To my father Julio César Guanziroli

Institutionen för klinisk vetenskap, intervention och teknik (CLINTEC), Enheten för kirurgi

Liver regeneration and function following portal vein occlusion and hepatectomy

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ABSTRACT

Background: Portal vein occlusion (PVO) and associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) are two strategies employed to render patients with unresectable liver tumours resectable by increasing the size of the future liver remnant (FLR). There is still limited knowledge about several aspects of PVO and most aspects of ALPPS.

Aims: To evaluate tumour progression after portal vein embolization (PVE) in patients with colorectal liver metastases (CRLM) treated with pre-procedural chemotherapy. To investigate if ALPPS performed after failed PVO in patients with CRLM treated with neoadjuvant chemotherapy is safe and feasible. To assess and compare liver volume and function in patients subjected to ALPPS. To study the levels of liver regenerative growth factors in ALPPS.

Methods: In paper I, patients with CRLM and response to neoadjuvant chemotherapy, subjected to PVE at Skåne University Hospital (2005-2013) and Karolinska University Hospital (2004-2010) were included in the study and assessed for tumour progression. In paper II, patients subjected to ALPPS after failed PVO at Karolinska University Hospital were included and efficacy and safety of the procedure in these patients was evaluated. In paper III, the liver volume and function in patients subjected to ALPPS was studied with a multimodal approach (including repeated computed tomography and hepatobiliary scintigraphy). In paper IV, sequentially sampled tissue from patients operated with ALPPS was analysed to assess the levels of liver regenerative growth factors.

Results: In paper I, 34 patients were identified and included. The median time between ended chemotherapy and PVE was 16 days. Three patients had tumour progression in the embolized liver lobe and three in the non-embolized lobe. Only two patients experienced tumour progression in the FLR that inhibited curative resection. In paper II, eleven patients operated with ALPPS after failed PVO were included. Six days after stage 1 the median growth of the FLR was 61.8% and all patients could proceed to stage 2 and resection of the liver tumours on day 7, with low morbidity and no 90-day mortality. In paper III, nine patients were studied and the increase in FLR-volume exceeded the increase in FLR-function at day 6 after stage 1, where functional increase only reached 50% of the volume increase in the FLR. In paper IV, ten patients were studied. The levels of HGF in plasma correlated with the degree of growth of the FLR and the levels of IL-6 correlated with the HGF levels.

Conclusions: The rate of progression of CRLM after PVE with pre-procedural chemotherapy is lower than previously reported if the time between the end of chemotherapy and PVE is short. The powerful growth of the FLR associated with ALPPS seems to be maintained in patients with CRLM treated with neoadjuvant chemotherapy and previously failed PVO. In the inter-stage period of ALPPS the high volume increase is not paralleled by a corresponding functional increase. The levels of HGF in plasma correlates with the degree of growth of the FLR and the levels of IL-6 correlates with the HGF levels in patients operated with ALPPS.

LIST OF SCIENTIFIC PAPERS

I. Spelt L, Sparrelid E, Isaksson B, Andersson R, Sturesson C
Tumor growth after portal vein embolization with pre-procedural chemotherapy for colorectal liver metastases
HPB (Oxford), 2015, 17(6):529-35

II. Sparrelid E, Gilg S, Brismar TB, Lundell L, Isaksson B
Rescue ALPPS is efficient and safe after failed portal vein occlusion in patients with colorectal liver metastases
Langenbeck's Arch Surg, 2016, Oct 19 (Epub ahead of print)

III. Sparrelid E, Jonas E, Tzortzakakis A, Dahlén U, Murquist G, Brismar TB, Axelsson R, Isaksson B
Dynamic evaluation of liver volume and function in associating liver partition and portal vein ligation for staged hepatectomy
Submitted manuscript

IV. Sparrelid E, Johansson H, Gilg S, Nowak G, Ellis E, Isaksson B
HGF levels correlates to the growth of the liver in patients operated with ALPPS
Submitted manuscript

CONTENTS

LIST OF ABBREVIATIONS

CRLM	Colorectal liver metastases
FLR	Future liver remnant
PHLF	Posthepatectomy liver failure
PVO	Portal vein occlusion
PVE	Portal vein embolization
PVL	Portal vein ligation
TSH	Two-stage hepatectomy
ALPPS	Associating liver partition and portal vein ligation for staged hepatectomy
ISGLS	International Study Group of Liver Surgery
TLV	Total liver volume
TV	Tumour volume
TFLV	Total functional liver volume
BW	Body weight
TELV	Total estimated liver volume
BSA	Body surface area
sFLR	Standardized FLR
ICG	Indocyanine green
HBS	Hepatobiliary scintigraphy
MRI	Magnetic resonance imaging
CE-CT	Contrast-enhanced computed tomography
RECIST	Response evaluation criteria in solid tumours
KGR	Kinetic growth rate
DPL	Deportalized lobe
EGF	Epidermal growth factor
VEGF	Vascular endothelial growth factor
TNF-alpha	Tumour necrosis factor alpha
IL-6	Interleukin 6
HGF	Hepatocyte growth factor

1 INTRODUCTION

Despite recent advances in both chemotherapy and ablation therapy, surgical resection remains as the gold standard for curative treatment of liver tumours [1, 2]. The development in liver surgery has been impressive during the last two decades which is attributed to several factors, such as improved surgical technique, optimized anaesthesia, modern chemotherapy drugs, refined radiology and methods for inducing preoperative growth of the healthy part of the liver [3]. One important feature, emanating from patients with colorectal liver metastases (CRLM), is a paradigm shift in assessing resectability. Where the old paradigm focused on mainly tumour related factors [4], the new paradigm instead defines resectability focusing on achieving radical resection with an adequate volume of the remaining liver (future liver remnant: FLR). Today the main restriction for curative liver resections is when the FLR is too small to sustain postoperative liver function during the regenerative process, as post-hepatectomy liver failure (PHLF) still is the main reason for mortality after liver surgery [5-9]. Therefore, together with the mentioned paradigm shift, there has been an increasing interest in developing different techniques that induce growth of the FLR, such as selective portal vein occlusion (PVO) by embolization (PVE) or ligation (PVL), two-stage hepatectomy (TSH) and more recently a new method called associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) [10-12]. These techniques aim at converting irresectable patients due to small FLR to candidates for resection by achieving sufficient growth of the FLR, thus to allow curative liver surgery without unacceptable risk of PHLF. While small FLR volumes correlates with risk for PHLF there is still a need for methods to evaluate liver function more accurately than with just volume measurement [13]. This has resulted in an increased interest in studying both liver regeneration and liver function in relation to these techniques and liver resection [14-18].

In summary there is only low-grade evidence for several topics in this field. The main reason is that these procedures have been applied to a limited number of patients without consistent definitions, making comparisons of studies difficult. The aim of the performed studies of this doctoral project is to contribute to the understanding of some key concepts regarding PVO and ALPPS.

2 BACKGROUND

2.1 FUTURE LIVER REMNANT

2.1.1 Partial hepatectomy as a model to study liver regeneration

Our knowledge of liver regeneration after partial hepatectomy is mainly derived from experimental models in rodents [19]. The most used model still consists of a 70 percentage hepatic resection in rats, developed by Higgins and Anderson already in 1931, and is one of the most studied models of tissue regeneration. As a result the understanding of the process of liver regeneration after partial hepatectomy has increased during the last decades. The role of certain cytokines and growth factors, such as interleukin 6, tumour necrosis factor-alpha, hepatocyte growth factor, epidermal growth factor, have been well characterized in corresponding experimental settings [20]. In humans there are studies investigating liver regeneration, mainly in healthy individuals undergoing living donor liver transplantation [21, 22]. In a study by Marcos et al [23] both donor and recipient in living donor liver transplantation were studied, and most of the regeneration in both the recipient and the donor occurred during the first week after resection or transplantation. However, most of the patients subjected to partial hepatectomy do not have healthy livers due to several causes (chemotherapy prior to surgery, cirrhosis, steatosis, cholestasis, among others) [2]. There have been sporadic reports on analysis of growth factors in patients with CRLM operated with partial hepatectomy [24], but little is known about how the temporal changes in growth factors are manifested before and after both stages of ALPPS. Again, our current knowledge of these potential mechanisms is based on some animal models [25, 26] and few on studies in humans [27, 28].

2.1.2 Posthepatectomy liver failure

PHLF is one of the most feared complications after liver surgery and is associated with substantial morbidity and mortality [29, 30]. It is suggested that PHLF is the direct, or contributing, cause behind the majority of fatalities after liver resection [30]. There is a large variability in the reported incidence of PHLF in the literature, mainly due to a lack of a uniform definition of this condition [31]. Currently there are three dominating criteria for defining and predicting PHLF: the ’50-50 Criteria’ [5], the ISGLS Definition [8] and the peak bilirubin > 7 [32]. This contributes to the many difficulties in comparing PHLF between different studies. The mechanisms involved in PHLF are known to be multifactorial and several risk factors have been identified. These risk factors can be divided into three categories: patient comorbidity, background liver disease and surgery related factors [31]. For patient related factors age [5], diabetes mellitus [33] and obesity [34] are associated with increased risk for postoperative mortality associated with PHLF. Liver related factors such as chemotherapy-associated liver injury [35, 36], cirrhosis [37, 38] and cholestasis [39, 40] are probably the most important conditions that can influence postoperative regeneration and contribute to increased risk for PHLF.

Since there still are no reliable regional liver function tests (in order to accurately assess preoperative function of the FLR), measured volume of the FLR together with the estimated quality of the background liver tissue is central for preoperative decision-making when assessing resectability in order to avoid PHLF [41]. To this date there is no effective way to treat PHLF once it has occurred, and the quandary hepatobiliary surgeons are faced with is the pursuit to offer an increased amount of patients a curative resection without a rise in potentially fatal PHLF.

2.1.3 Volume assessment of the future liver remnant

FLR can be evaluated volumetrically in several ways. The original method consists in radiological measurement of the FLR volume and total liver volume (TLV) without tumours by subtracting tumour volume (TV), to obtain a total functional liver volume (TFLV) [42-44]. The FLR volume (also with exclusion of eventual tumours) is then divided by the TFLV and multiplied with 100. This provides a percentage on how large portion of the total liver is represented by the FLR, and the formula for calculating FLR% = FLR / TFLV *100. This method requires an extensive and sometimes complicated volumetric measurement which is the rationale behind the development of the subsequent methods for evaluating the size of the FLR.

Another method, originating from the living donor liver transplantation setting [45, 46], is to relate the volume of the FLR to body weight (BW) [47]. Since body weight correlates well with the weight of the liver [48], and ml/cm^3 from the radiological measurements correlates with the weight of the liver in mg, body weight in mg can be translated to ml according to the formula: FLR/BW% = FLR / BW (kg x 1000) *100.

A third, more recent, method uses a formula to calculate the total estimated liver volume (TELV) with body surface area (BSA) and is based on autopsy studies (TELV = -794 + 1276 x BSA) [48-50]. This can then be used to calculate a standardized FLR (sFLR), also expressed as percentage, by the formula sFLR% = FLR / TELV *100.

There is currently no consensus on which method that should be used, causing difficulties in interpreting results from different studies involving evaluation of the FLR [51-53]. In addition, there is no consensus on the required size of the FLR that should be obtained in order to avoid PHLF. Traditionally a FLR of 30% or more was considered sufficient for safe resection [54]. Currently, when evaluating the FLR before resection for CRLM there is a variety in recommended threshold levels, but most consider a FLR between 25-30% to be sufficient if previous chemotherapy have been administered to the patient [55, 56]. In patients with perihilar cholangiocarcinoma or hepatocellular cancer generally a larger FLR is considered to be necessary for safe resection due to a more impaired function of the native liver, but also here the variability applied complicates adequate comparison [57-59]. Also when FLR/BW is used there is a similar situation with different levels (between 0.4-0.8% for CRLM) being used for deciding on resectability [47, 51].

2.1.4 Function assessment of the future liver remnant

Several attempts to characterize FLR function instead of mere volume have been presented [60-66]. These consist mainly of biochemical parameters, ICG clearance test, hepatobiliary scintigraphy (HBS) and dynamic MRI. ICG clearance test is an established method to evaluate global liver function and is mainly used for preoperative assessment of liver function [43, 57, 67, 68]. However, it lacks the capacity to characterize regional function within the liver. Studies on HBS after PVE have shown that FLR function precede the volume increase after PVE [69, 70]. In this context it should be mentioned that HBS might even be more reliable than volume-based data in predicting PHLF [71], and a cut-off value for safe resection based only on HBS has been suggested [72].

Still, volumetric evaluation of the FLR together with consideration for patient factors and assumed quality of the background liver, remain as largely dominating.

2.2 PORTAL VEIN OCCLUSION

PVO consists of a selective occlusion of the portal vein to the tumour bearing part of the liver, diverting all portal blood to the FLR in order to induce hypertrophy of this part. This can be achieved by both PVE and PVL, and the effect is then evaluated with radiological volume analysis, generally with contrast enhanced four-phase computed tomography (CE-CT) [73-78]. There are several controversies regarding PVO.

(i) *PVE vs. PVL*

There are no randomized controlled studies comparing PVE to PVL. The main reason for this is that when you have a tumour-free FLR the option to perform a less invasive PVE at the angiography suite will make most liver surgeons reluctant to even consider PVL in these patients. While on the other hand many centres prefer PVL, instead of PVE, as part of an operation to clear the FLR from tumour prior to a second operation (i.e. TSH). A recent meta-analysis states that there is no evidence of an obvious advantage of one technique over the other [79], although one has to bear in mind that all nine studies included in that meta-analysis where small case series with heterogeneous design in terms of for example PVO technique, patient cohorts and waiting time to CE-CT.

(ii) *PVE technique*

Some argue that in PVE, embolization technique (extending embolization to include segment IV) and material (using histoacryl instead of microparticles) can improve the results [80, 81]. Here the evidence grade again is poor and based on single centre case series [82]. A head to head comparison between PVE and PVL is still lacking.

(iii) *Tumour progression after PVO*

Also when it comes to risk for tumour progression after PVO result are conflicting. Most agree that there is a risk for progression while waiting for the effect of the PVO, but there is a variance in risk in the presented studies [83, 84]. When performing PVO in patients with CRLM they have in general been subjected to neoadjuvant pre-procedural chemotherapy. Some argue that there is a need for chemotherapy after PVE to prevent progression [85, 86], others that this risk is small if the time from ending pre-procedural chemotherapy to PVE is kept short [87]. One recent study reports a high risk for tumour progression after PVE [88].

(iv) *Inhibitory factors for hypertrophy after PVO*

Several factors are considered to impair the growth of the FLR after PVO. Among these are pre-procedural chemotherapy, high bilirubin levels, concomitant cholangitis and diabetes mellitus [76, 89], but the results are still conflicting [90].

(v) *Alternatives after insufficient effect of PVO*

PVO fails to achieve sufficient growth of the FLR in a considerable amount of patients leaving them with no other option than palliative chemotherapy at best [91]. Despite additions to PVE, mainly with hepatic vein embolization [92], this fact remains.

2.3 TWO-STAGE HEPATECTOMY

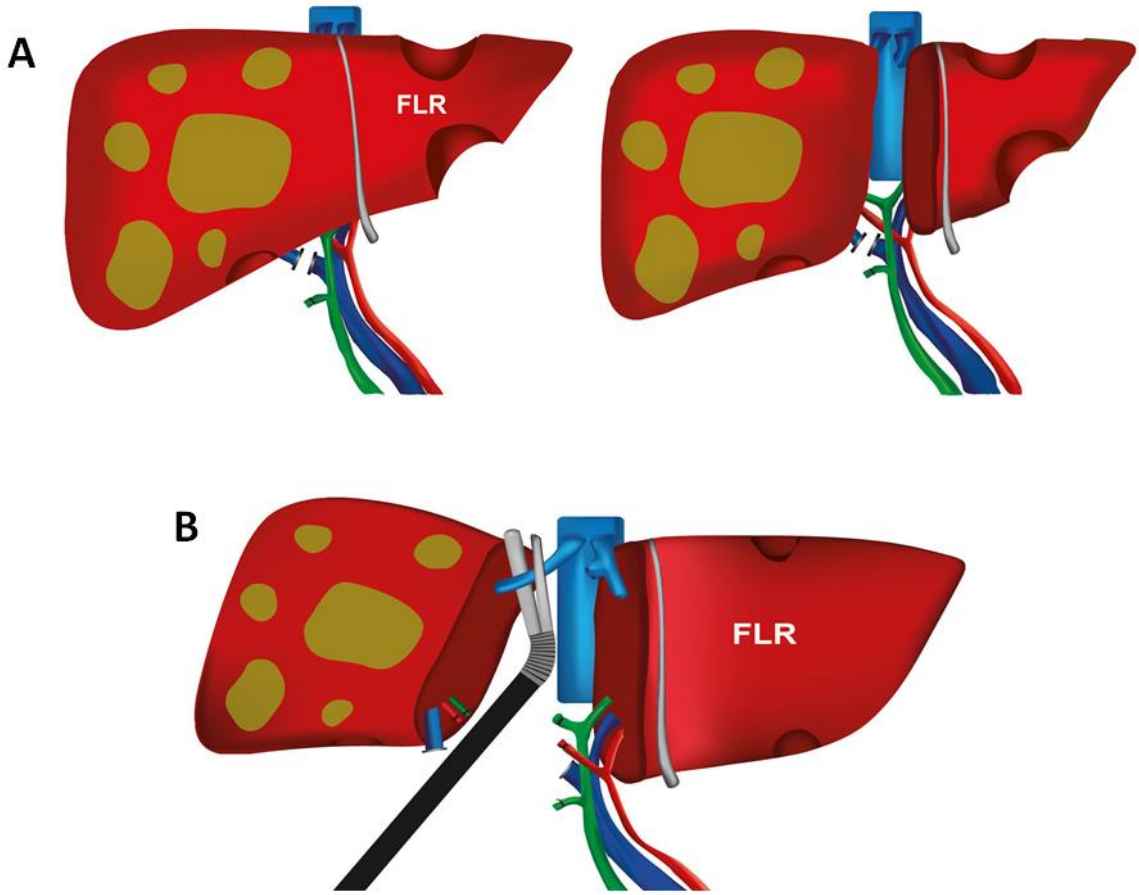
TSH takes advantage of the regenerative capacity of the liver with tumour clearance of the FLR in a first operation. After a waiting time allowing for the FLR to regenerate, a second operation with a larger liver resection is performed [93, 94]. TSH has then almost exclusively been combined with PVO to induce faster and larger hypertrophy of the FLR [11]. The main drawback with TSH (from now together with PVE or PVL) is that a considerable amount of patients either experience insufficient growth of the FLR or experience tumour progression while waiting for the effect of the PVO [95]. Some argue that this is actually a test of time for these patients, in that the time from PVO to CE-CT will offer a better selection of the patients that will benefit from surgery by excluding the ones that experience tumour progression while waiting [96]. Even if this might be these aspects have been questioned [97], and furthermore do not apply to the patients that experience insufficient effect of the PVO without tumour progression. Modern chemotherapy has extended median survival for patients with CRLM treated with palliative chemotherapy to a median of more than 20 months and five year survival in the range of 10% [98, 99], a fact that has to be taken into consideration. Still resection is the only curative treatment and for other diagnosis than CRLM chemotherapy options are limited or not as beneficial. It is in the light of this that new conversion therapies are being received with great interest.

2.4 ALPPS

Associating liver partition and portal vein ligation with staged hepatectomy (ALPPS) was first described systematically in a multicentre study from Germany in 2012 [12]. In this study 25 patients were subjected to a new procedure combining selective right-sided portal vein ligation with parenchymal transection between the tumour bearing liver (typically segment IV-VIII) and the FLR (segment I-III) in the stage one operation. Following a very fast and powerful hypertrophy of the FLR, the second stage was performed after a median of nine days where the tumour bearing liver was removed by dividing the right bile duct, liver artery and liver vein (the ALPPS procedure is illustrated in **Figure 1** below). This retrospective analysis included patients from five regional hospitals and consisted of several types of both primary and secondary liver tumours. The unprecedented growth rate of the FLR created substantial attention from the hepatobiliary surgical community. Together with this attention there was also concern about the high morbidity and mortality presented in the study and a discussion about the oncological rationale behind this new procedure [96, 100-103]. The initial study was immediately followed by several reports of which most contained personal communications rather than original articles. To this date almost 200 publications about ALPPS can be found in PubMed covering less than 5 years, but only merely 50 of these are original articles (when case reports, letters to the editors, reviews, etc. are excluded) being mainly small single centre series.

The original study by Schnitzbauer et al was followed by the experience from other centres, reporting a maintained high morbidity and mortality [104-107]. When analysing the patients suffering from major complications and mortality after the procedure, there was a clear pattern indicating that patients with concomitant biliary surgery were overrepresented. In the first publication from the International ALPPS Registry [108] these observations were confirmed, and it was also shown that when ALPPS was performed on patients with CRLM morbidity and mortality was significantly lower. This has been further investigated and confirmed in later publications [97, 109-114]. The proponents of TSH with PVO claimed that this strategy remained equally efficient as ALPPS [115, 116] but without taking into account that up to 30% of the patients never completed the two stages compared to a completion rate of 97-100% with ALPPS [97]. Further criticism was directed towards ALPPS due to preliminary reports on early tumour recurrence [117]. In this context it is conceivably not appropriate to compare with standard liver resections. ALPPS is probably performed in more advance tumour situations where we, prior to the availability of ALPPS, could not offer a curative alternative. Consequently patients subjected to palliative chemotherapy might be the appropriate group to compare with [118]. However, there is still very limited survival analysis following ALPPS so this question remains to be answered.

Figure 1. *The ALPPS procedure illustrated.*



(A) Division of the right portal vein and parenchymal transection, together with eventual tumour clearance of the FLR, performed at stage 1. (B) After adequate hypertrophy of the FLR, stage 2 is performed with division of right pedicle and liver vein.

(Modified with permission from Eduardo de Santibañes.)

Considering the high morbidity and mortality associated with ALPPS, many advocate to restrain this procedure mainly to patients with CRLM. In addition, there are also proponents for the continued use of PVO as the primary method for augmenting FLR size, while recognizing ALPPS as a rescue option previously not available for patients with insufficient effect on the FLR after PVO [119]. This version of ALPPS, has been named rescue ALPPS (where only parenchymal transection is being performed in stage 1 if previous PVO consists of PVL). There are a few reports about this procedure, containing only a few patients each, where equivalent growth of the FLR as in upfront ALPPS was seen despite previous PVO [105, 107, 120-122]. In this context the question arises whether patients with very small FLR should be considered for ALPPS upfront. Some patients cannot be expected to experience a sufficient effect of PVO considering the more moderate response to this procedure when compared to ALPPS. Hence waiting for the effect after PVO when the chance of achieving a sufficient effect on FLR is almost non-existing might be an argument in favour of ALPPS

upfront. The potent effect ALPPS has on the FLR has even enabled resecting all but one liver segment in selected cases [118].

The mechanisms behind the potent growth of the FLR that the first stage of ALPPS induces are still unknown. However, two theories predominate. One highlights the division of the parenchyma that disrupts eventual collaterals between the FLR and the tumour bearing liver, thus directing all portal blood to the FLR and thereby facilitating increased hypertrophy [123]. This theory has not been investigated in detail and still remains speculative. Another theory suggests that the tissue damage caused by the parenchymal transection might release a humoral response leading to faster and greater growth of the FLR [25]. Finally studies about the function of the FLR after ALPPS are almost completely lacking. The previous studies on PVE where function preceded volume increase might not be applicable to the ALPPS setting.

In conclusion, there is a continued need for studies enabling us to understand the central mechanisms behind the clinical effects of both PVO and ALPPS. For both techniques improved evaluation of the FLR remains essential. Our ambition with the presented studies is that they will contribute to this.

3 AIMS

The aims of this thesis were:

Aim I: To evaluate tumour progression after portal vein embolization in patients with colorectal liver metastases treated with pre-procedural chemotherapy.

Aim II: To investigate if ALPPS performed after failed portal vein occlusion in patients with colorectal liver metastases treated with neoadjuvant chemotherapy is safe, feasible and effective.

Aim III: To assess and compare liver volume and function in patients subjected to ALPPS.

Aim IV: To study the levels of liver regenerative growth factors before and after both stages of ALPPS and to analyse if the grade of hypertrophy in the future liver remnant is influenced by these growth factors.

4 METHODS

4.1 PATIENTS

In **Paper I** patients subjected to PVE between 2005 and 2013 at Skåne University Hospital, Lund, Sweden, and between 2004 and 2010 at Karolinska University Hospital, Stockholm, Sweden, were identified. Of those, patients undergoing a PVE before an intended resection for CRLM were identified, including only patients who received chemotherapy within two months before PVE, resulting in at least stable tumour situation according to RECIST 1.1 criteria [124].

In **Paper II** patients with CRLM previously treated with neoadjuvant chemotherapy and subjected to PVO but with insufficient effect on the FLR were eligible for the study. A FLR/BW of less than 0.5% was considered as an indication for ALPPS after failed PVO [47].

In **Paper III** and **IV** patients with CRLM that responded to neoadjuvant chemotherapy and could be rendered tumour free by an extended right-sided hemihepatectomy (segments IV-VIII) but where the FLR/BW was below 0.5% were considered for inclusion in the study. Patients with previous PVO (PVE or PVL) with insufficient response to the procedure - still with a FLR/BW of less than 0.5% - where also considered eligible for inclusion in the study if they fulfilled the inclusion criteria stated above.

4.2 EVALUATION OF TUMOUR PROGRESSION AFTER PVE

In **Paper I** CE-CT investigations were collected retrospectively and assessed with detailed volumetry. The images used for this were the most recent CE-CT prior to initiating pre-procedural chemotherapy, the most recent scan before PVE (generally the CE-CT for evaluating the effect of chemotherapy), and the CE-CT used to evaluate the effect of PVE. The liver metastases were considered as bilobar or unilobar depending on their locations. Metastases located between both liver lobes were defined as either left or right sided depending on where its geometrical centre was located. TFLV was obtained by subtracting TV from TLV. FLR was measured as for an intended extended right sided hemihepatectomy, meaning that the FLR volume consisted of segments I-III (without eventual tumours in the FLR) in all patients. According to the RECIST 1.1 criteria two target lesions were assessed in each liver lobe, except in cases of solitary metastases or unilobar manifestation. Tumour growth was determined by measuring changes in maximum diameter of the target lesions between the analysed scans. Characterization of tumour progression between two CE-CT was made for the right and left hemi-liver respectively, and classified as complete response, partial response, stable disease or progressive disease.

In addition, the total volume of all metastases in the right and left lobe, respectively, was calculated in the three CE-CT of each patient, by outlining the tumours in each transversal image to calculate the area, adding up all calculated areas from the different slides and then

multiplying by slide thickness, which was 5 mm or less. As described above, metastases located between both liver lobes were defined as either left or right sided depending on where its geometrical centre was located, hence the tumour volume of these lesions were assigned to only one lobe. Alterations in tumour volume could then be calculated. Analyses were made to compare tumour progression between the right and the left lobe, hence comparing tumour progression between the embolized and non-embolized liver lobe. Tumour progression in right-sided metastases was compared with left-sided metastases within the entire population of both patients with bilobar manifestation, as well as those with unilobar metastases.

4.3 PORTAL VEIN OCCLUSION PRIOR TO ALPPS

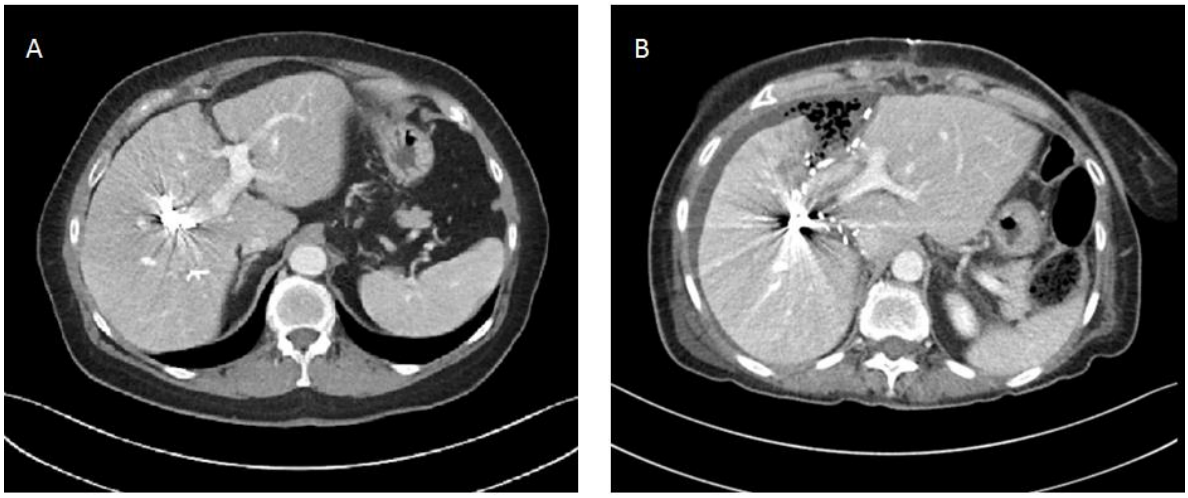
PVE was performed with percutaneous ipsilateral technique and puncture of peripheral portal branches of the right side. Polyvinyl alcohol beads (Terumo Bead Block™ Embolic Bead, Metron Healthcare, Athens, Greece) and polyvinyl alcohol particles (Contour™, Boston Scientific, Cork, Ireland) were combined with central coils (MicroNester® Embolization Coil, Cook, IN, USA), placed in the right portal vein, to obtain occlusion of the portovenous system to segment V-VIII. The portal branches to segment IV were not embolized as this was not routine at our centre at the time of the studies.

PVL was performed by dividing the right portal vein with a stapler instrument (Endo GIA™ Universal with Tri-Staple™, Covidien, Dublin, Ireland). PVL was preferred over PVE only when the FLR contained metastases and used together with local resections in the FLR at the stage 1 operation of an intended conventional TSH.

4.4 SURGICAL STRATEGY AND ASSESSMENT OF COMPLICATIONS

ALPPS was performed in a similar way as described previously [12]. The patients with failed PVE were also subjected to ALPPS with surgical division of the right portal vein (an example of rescue ALPPS after failed PVE can be seen in **Figure 2** below). In rescue ALPPS after failed PVL only parenchymal transection was performed at stage 1 since the right portal vein was already divided. If volumetry performed on the CE-CT on day 6 after stage 1 showed sufficient hypertrophy of the FLR (resulting in a FLR/BW >0.5%), the stage 2 procedure was performed on day 7. At stage 2 the right portal pedicle and right liver vein was divided using a stapler instrument and the tumour bearing deportalized liver could be removed as in an extended right-sided hemihepatectomy preserving only segment I-III in all patients. In case of metastases in the FLR these were resected when possible, or microwave ablated if located deep in the FLR. Irrespectively of technique, tumour clearance of the FLR was performed during the stage 1 procedure.

Figure 2. *Exampel of rescue ALPPS after failed portal vein embolization*



CE-CT after PVE (A) and CE-CT before stage 2 (B) in the same patient.

Procedure-related complications were assessed according to the Clavien-Dindo classification of surgical complications [125, 126]. Evaluation of PHLF was made using the three dominating criteria for defining and predicting PHLF: '50-50 Criteria', ISGLS Definition and peak bilirubin >7 [5, 8, 32].

4.5 VOLUMETRIC ASSESSMENT IN ALPPS

Pre-PVO and preoperative FLR volume was calculated from four-phase CE-CT of the liver. In **Paper II** the CE-CT evaluating the effect of the PVO was performed after 28 days in median (range 19-33) and was used as baseline investigation. To measure the effect on the FLR in ALPPS all patients underwent a new CE-CT of the liver six days after stage 1. In **Paper III** and **IV** an additional low-dose CT was performed at day 7 and 28 after stage 2 to assess the size of the liver remnant. FLR/BW was calculated and TELV was calculated according to the previously described formula developed by Vauthey and co-workers to obtain sFLR [50]. Percentage FLR increase on day 6 after stage 1 and days 7 and 28 after stage 2 examinations were calculated with the preoperative FLR as reference. The kinetic growth rate (KGR) of the FLR was calculated separately for the three time intervals (between the stage 1 and 2 operations, for the first seven days after stage 2 and for days 8-28 after stage 2) by dividing the percentage increase of the FLR by the number of elapsed days between the examinations, and was then expressed as percentage change per day [115]. All calculations of liver volume was performed by using the software Volume Viewer© (Voxtool 11) for AW Volume Share 5 implemented on an AW Workstation (GE Healthcare, Fairfield, CT, USA).

4.6 EVALUATION OF LIVER FUNCTION IN ALPPS

4.6.1 Indocyanine green clearance

Indocyanine green clearance (ICG-C) measured as plasma retention at 15 minutes, expressed as percentage (ICG-R15%) was performed at six time points using the LiMON® system (PULSION Medical System, Munich, Germany) after intravenous injection of 0.5 mg/kg of ICG dye (Verdye®, Diagnostic Green GmbH, Aschheim-Dornach, Germany). The time points for the ICG-C tests were the day before stage 1 (D-1 OP1), the day after stage 1 (D1 OP1), the day before stage 2 six days after stage 1 (D6 OP1), the day after stage 2 (D1 OP2), seven and 28 days after stage 2 (D7 OP2 and D28 OP2).

4.6.2 Serum liver function tests

Prothrombin time measured as the international normalized ratio (INR) and serum bilirubin levels were measured daily from the day before stage 1 until day 7 after stage 2 and then again on day 28 after stage 2.

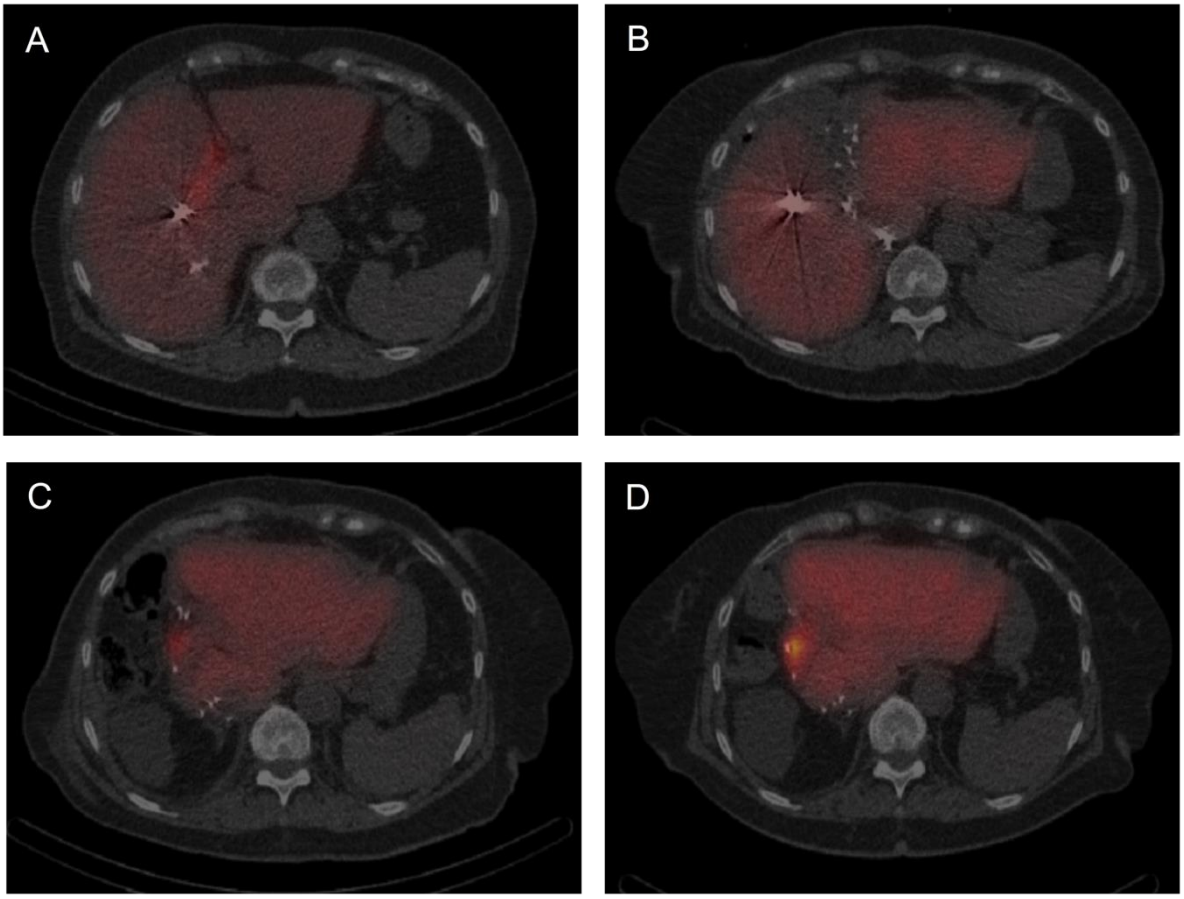
4.6.3 Hepatobiliary scintigraphy

Hepatobiliary scintigraphy and calculation of functional parameters were performed according to a method described previously [70, 127]. Without repositioning the patient a low-dose non-contrast-enhanced CT was performed for attenuation correction and anatomical mapping. Data were processed on a Hermes workstation (Hermes Medical Solutions AB, Stockholm, Sweden).

Total liver ^{99m}Tc-mebrofenin uptake rate (%/min), representing total liver function (TL-function), was calculated from the dynamic acquisitions as geometric mean. To compensate for differences in individual metabolic requirements, TL-function was divided by body surface area (BSA) according to the Mosteller formula and expressed as %/min/m^2. For calculation of the FLR-function a region of interest (ROI) delineating the FLR was drawn. On the preoperative examination the falciform ligament/umbilical fissure, as visible on anterior CT projections was used to delineate the border between segments II/III and IV. On day six after stage 1 the FLR was well demarcated by the crevice between segments II/III and segment IV facilitating drawing of ROI's of the FLR as well as the deportalized lobe (DPL) for calculation of both FLR-function and DPL-function. Calculation of FLR-function and DPL-function was done by dividing the added counts 150-350 seconds after isotope injection within the respective delineated ROI's by the total liver counts within the same time frame and multiplying this factor by the total liver ^{99m}Tc-mebrofenin uptake rate with values expressed as %/min/m^2. Mebrofenin uptake per litre of FLR tissue was calculated by dividing the FLR-function (not corrected for BSA) by FLR-volume and expressed as %/min/l. Increase in FLR-function on day 6 after stage 1 and on day 7 and day 28 after stage 2 were calculated with the preoperative FLR-function as reference and expressed as percentage increase. Kinetic growth rate (KGR) in FLR-function was calculated for the three time intervals (between the stage 1 and 2 operations, during the first seven and days 8-28 after

stage 2) by dividing the percentage increase for each time period with the number of elapsed days and expressed as percentage increase per day.

Figure 3. *Hepatobiliary scintigraphy at the four time-points.*



HBS with SPECT/CT in a patient with insufficient growth after previous PVE before stage 1 (A), on day 6 after stage 1 (B), day 7 (C) and day 28 (D) after stage 2.

4.7 TISSUE SAMPLING

10 ml peripheral blood was collected in an EDTA-coated tube at six time points; the day before stage 1 (D-1 OP1), the day after stage 1 (D1 OP1), the day before stage 2 six days after stage 1 (D-1 OP2), the day after stage 2 (D1 OP2), day 7 and 28 after stage 2 (D7 OP2 and D28 OP2). To obtain plasma the blood was centrifuged for 10 minutes at 4000 rounds per minute at 4°C (Hettich Zentrifugen Universal 320 R, Hettich Lab Technology, Tuttlingen, Germany). Plasma was stored in 2 ml micro tubes (Sarstedt AG & Co, Nürnbrecht, Germany) at -80°C until analysed. Biopsies from the left and right liver lobe (segment III and V) where taken at both stages of ALPPS using biopsy needle (True-Cut™ Biopsy Needle, CareFusion, Sheffield, UK). The biopsies were directly transferred into liquid nitrogen in 2 ml cryogenic vials (Corning®, Corning Inc, NY, USA) and stored at -80°C until analysed.

4.8 QUANTIFICATION OF PLASMA MARKERS AND MRNA

Plasma levels of epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), tumour necrosis factor-alpha (TNF-alpha) and interleukin 6 (IL-6) were measured in triplicate using a Luminex bead-based multiplex assay. Briefly, the assay relies on multi-coloured magnetic beads coated with antibodies. Plasma hepatocyte growth factor (HGF) concentrations were determined in triplicates with a solid phase sandwich enzyme-linked immunesorbent assay (ELISA). Total mRNA was extracted from the liver biopsies using Trizol reagent. Quantification of mRNA was performed using real time PCR. All samples were analysed in triplicates. Relative mRNA expression was calculated.

4.9 STATISTICAL ANALYSIS

4.9.1 Paper I

Statistical analysis was made with SPSS® Statistics, version 21 (IBM, Chicago, IL, USA). Median values with range were used for continuous variables whereas frequencies were calculated for categorical variables. RECIST classification was compared between groups and specifically the percentage of cases that showed progressive disease. Fisher's exact test was used to test for statistical significance. The tumour volume change as described above was compared between groups using Mann-Whitney *U* test. Correlation between tumour growth rate and time from end of chemotherapy to PVE was calculated using linear regression analysis and computing a Pearson correlation coefficient. P-values of <0.05 were considered to represent statistical significance.

4.9.2 Paper II

Statistical analysis was made using JMP® version 5.1 (SAS Institute Inc, Cary, North Carolina, USA). Median values with range were used for continuous variables whereas frequencies were calculated for categorical variables. Paired t-test was used to compare means between different time points in the same patient. P-values of <0.05 were considered to represent statistical significance.

Since all variables in the study where presented as median (due to non-normally distributed values) it would have been appropriate to use a non-parametric test, instead of the parametric paired t-test. However, the statistical tests in the study were controlled (after publication) using a non-parametric Wilcoxon signed-rank test, with maintained statistical significance.

4.9.3 Paper III

Statistical analysis was performed using SPSS® Statistics, version 23 (IBM, Chicago, Illinois, USA) and GraphPad Prism®, version 7 (GraphPad Software, La Jolla, California, USA). Median values with range were used for continuous variables whereas frequencies were calculated for categorical variables. The Wilcoxon signed-rank test was used to compare differences in liver volume and function and the Spearman's rank correlation coefficient was

used to test for correlation between volumetric and functional parameters. Two-tailed P-values of <0.05 were considered to represent statistical significance.

4.9.4 Paper IV

Statistical analysis was performed using SPSS® Statistics, version 23 (IBM, Chicago, IL, USA) and GraphPad Prism®, version 7 (GraphPad Software, La Jolla, CA, USA). Continuous variables were expressed as median with range or inter-quartile range (IQR), whereas frequencies were calculated for categorical variables. Wilcoxon's signed rank test was used to compare differences in liver volume at different time-points, for comparison of growth factors between baseline and the different time-points and for mRNA expression between stage 1 and 2 of ALPPS. Correlations were evaluated with linear regression and Spearman's rank correlation. A two-tailed p-value of <0.05 was considered to represent statistical significance.

4.10 ETHICAL CONSIDERATIONS

The first study was approved by the Regional Ethical Review Board in Lund and in Stockholm, Sweden. Since this study was purely retrospective in nature, informed consent from the included patients was not required by the Regional Ethical Review Boards.

Studies two, three and four were approved by the Regional Ethical Review Board, Stockholm, Sweden. For study three approval was also granted from the Radiation Safety Committee at Karolinska University Hospital, Stockholm, Sweden. Since these studies were designed in a prospective manner written and oral informed consent was obtained from each patient before inclusion and the study protocol confirmed with the ethical guidelines of the 1975 Declaration of Helsinki.

5 RESULTS AND DISCUSSION

5.1 AIM I: ASSESSING TUMOUR PROGRESSION AFTER PVE

In **Paper I** a total of 34 patients with CRLM treated with chemotherapy and then right-sided PVE for an intended curative resection were included in the study. 23 patients had bilobar liver metastases. All patients had response to chemotherapy prior to PVE with a median of seven administrated cycles. Of the 34 included patients, 17 were subjected to an extended right-sided hemihepatectomy and nine to a right-sided hemihepatectomy. Eight patients were not resected due to extrahepatic tumour progress (n=3), insufficient growth of the FLR after PVE (n=3), pronounced progress of metastases in both liver lobes (n=1) and one new unresectable metastasis detected intra-operatively in the FLR (n=1).

Tumour progress in the right lobe was analysed for all patients (n=34) and tumour progress in the left lobe was analysed in patients with bilobar disease (n=23). There was no difference in tumour growth between the embolized and non-embolized liver lobe after PVE. There was a linear correlation between the volumetric tumour growth and time between end of chemotherapy and PVE ($r=0.25$, $p<0.001$).

In three patients progressive tumour growth was noted after PVE in both the embolized and non-embolized liver lobe, representing 3/34 and 3/23 of cases, respectively ($p=0.677$).

In this study we demonstrated a lower probability of progressive tumour situation after PVE compared to previous reports. Tumour response after PVE has been investigated previously to a limited extent and only one study [85] has evaluated tumour response after PVE according to RECIST criteria. In that study, Fischer et al reported a tumour progression rate of 34% after PVE. However, in the mentioned study, no details are stated about chemotherapy before PVE, making it difficult to compare these results.

With measurement of tumour volume we could demonstrate a decrease in tumour volume after PVE in both liver lobes (16% in right-sided tumours and of 11% in left-sided tumours), which is less than in previous studies. In a study by Simoneau et al [128] consisting of a large cohort of patients treated with chemotherapy and then subjected to PVE, a significant tumour growth after PVE was found. However, in the mentioned study the interval from the cessation of chemotherapy to PVE was 4 weeks compared to 16 days in our study. In another study by Pamecha et al [86] an increase in tumour diameter after PVE was reported. All patients in this study received pre-PVE chemotherapy, but details about chemotherapy response or time between the cessation of chemotherapy and PVE was not stated, making comparison between that study and ours difficult.

We are not the first ones to report tumour regression after chemotherapy and subsequent PVE. In a study by Pommier et al [129] tumour shrinkage of 4% in the right lobe and 9% in the left lobe was reported in patients with a ‘fast response’ to chemotherapy before PVE. In that study, the time between ending chemotherapy and PVE was also longer compared to our

study, again giving a possible explanation to the differences between the studies. The present study showed that tumour growth increases the longer the time elapsed between end of chemotherapy and PVE.

Chemotherapy prior to PVE has been suggested to reduce the effect of the procedure on the hypertrophy of the FLR. Prolonged pre-PVE chemotherapy has been associated with reduced hypertrophy in the non-embolized lobe [89, 130], but other studies have failed to show any influence of chemotherapy on regeneration [131, 132]. In the present study of right-sided PVE, the median FLR increased from 19% to 29%, which is similar, although somewhat lower, to what has been reported in previous studies [89, 132].

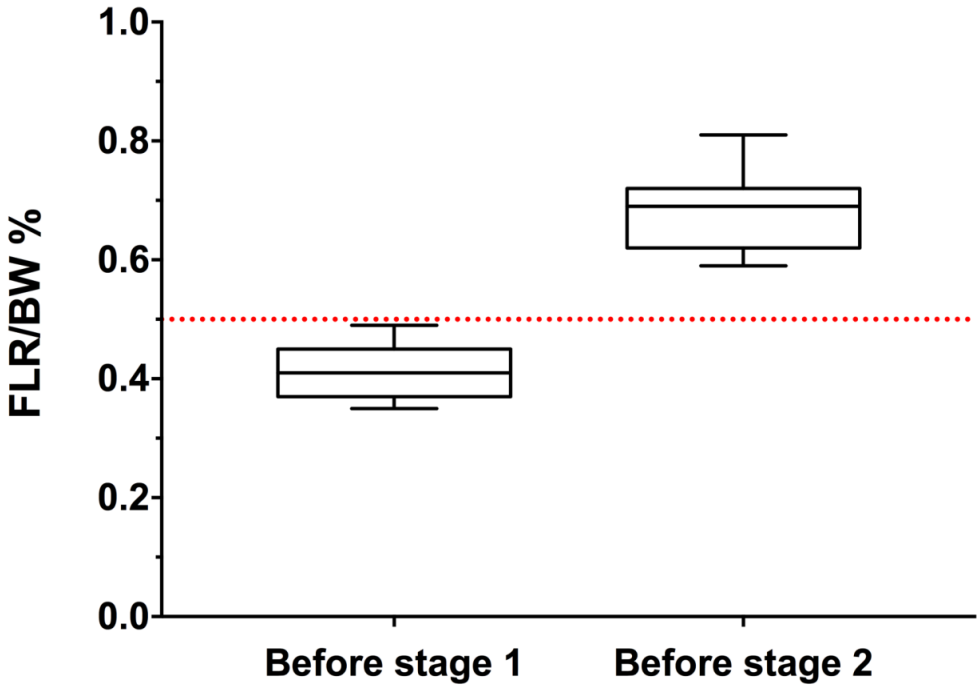
The results of the present study suggest that tumour growth in the right and left liver lobe after PVE is comparable. This is in accordance with two previous studies investigating the subject [85, 129].

5.2 AIM II: THE VALUE OF RESCUE ALPPS AFTER FAILED PVO

In **Paper II** eleven patients with CRLM responsive to neoadjuvant chemotherapy and then a PVO with insufficient effect (FLR/BW still below 0.5%) were included and submitted to ALPPS. The patients had a median of seven cycles of chemotherapy prior to PVO. Six days after stage 1, FLR was evaluated with CE-CT and stage 2 was performed on day 7. All patients had metastases located in both liver lobes (in the right lobe and in segment IV), but only four had metastases in segments I-III requiring tumour clearance in the FLR at the first stage of the ALPPS procedure. All patients completed stage 2 with radical (R0) removal of all metastases together with the deportalized liver (segments IV-VIII). The complication rate was low. Four patients had grade 3a-complications due to pleural effusion that was drained in local anaesthesia. There were no complications equal to or above grade 3b. Postoperatively no patient fulfilled the ’50-50 Criteria’, peak bilirubin >7 or ISGLS grade C for severe postoperative liver failure and no 90-day mortality occurred.

FLR volume before PVO was 250 ml (range 180-370). After PVO the FLR increased to 312 ml (range 260-450), representing a PVO induced hypertrophy of the FLR of 26.8% (range -7.3-66.7, p=0.006). Despite previous PVO there was an adequate hypertrophy of the FLR after the ALPPS stage 1 procedure. Six days after stage 1 the median volume increase of the FLR was 209 ml (range 87-314, p <0.001). This corresponded to an increase of FLR/BW to 0.69% (range 0.59-0.81), i.e. more than 0.5% in all patients (see **Figure 4**). The growth of the FLR between stage 1 and 2 was in median 61.8% (range 19.3 – 120).

Figure 4. *Increase in future liver remnant to body weight ratio.*



Box-whisker plot displaying the increase in FLR/BW between stage 1 and 2 of ALPPS in patients subjected to rescue ALPPS. Dotted red line shows 0.5%.

This study confirms results from previous reports that ALPPS can be safe, feasible and effective as a rescue procedure in patients with CRLM and insufficient effect on the FLR after previous PVO. In addition it seems as if the described high morbidity and mortality associated with ALPPS does not apply when performing the procedure in this clinical setting. Despite the limited number of patients enrolled, the patient cohort of this study still represents the largest series of rescue ALPPS for CRLM so far presented. The main aim of this study was, however, to specifically investigate the safety, feasibility and efficacy of rescue ALPPS in this clinical situation. The studied patients represent a comparatively homogenous cohort in that they all received chemotherapy for CRLM only, they were all subjected to PVO prior to ALPPS and they were all operated with an extended right-sided hemihepatectomy on the seventh day after stage 1 operation.

In the current study cohort the overall median growth of the FLR before stage 2 was 61.8%, which is less than reported in most previous studies. This might, however, be explained by two factors. Firstly, in contrast to many other reports on ALPPS, only patients with CRLM treated with pre-procedural chemotherapy were included. It is recognized that pre-PVE chemotherapy can have a negative effect on growth of the FLR after PVE [89, 133] and it might have the same effect after ALPPS, although this remains to be proven. Another complicating factor was that the interval between the cessation of chemotherapy and ALPPS was quite long due to the waiting time from PVO to ALPPS. Consequently the alleged negative effect of pre-procedural chemotherapy on FLR hypertrophy might have been less

pronounced in these patients compared to the patients undergoing ALPPS without previous PVO. Secondly, the inter-stage time of seven days used for all patients in this study was shorter than in most published series [111]. That might in part explain the somewhat less pronounced hypertrophy rate. On the other hand, the growth of the FLR of 61.8% in six days represents a kinetic growth rate of 10.3%/day which is higher than for example in the publication by Schnitzbauer et al [12] (74% increase in nine days = 8.2%/day). Of course, these calculations are based on the assumption that the present patients would have had a continued similar growth rate beyond the sixth day. Nevertheless, previous PVO does not seem to significantly impair the potent hypertrophic effect on the FLR induced by the stage 1 procedure in ALPPS.

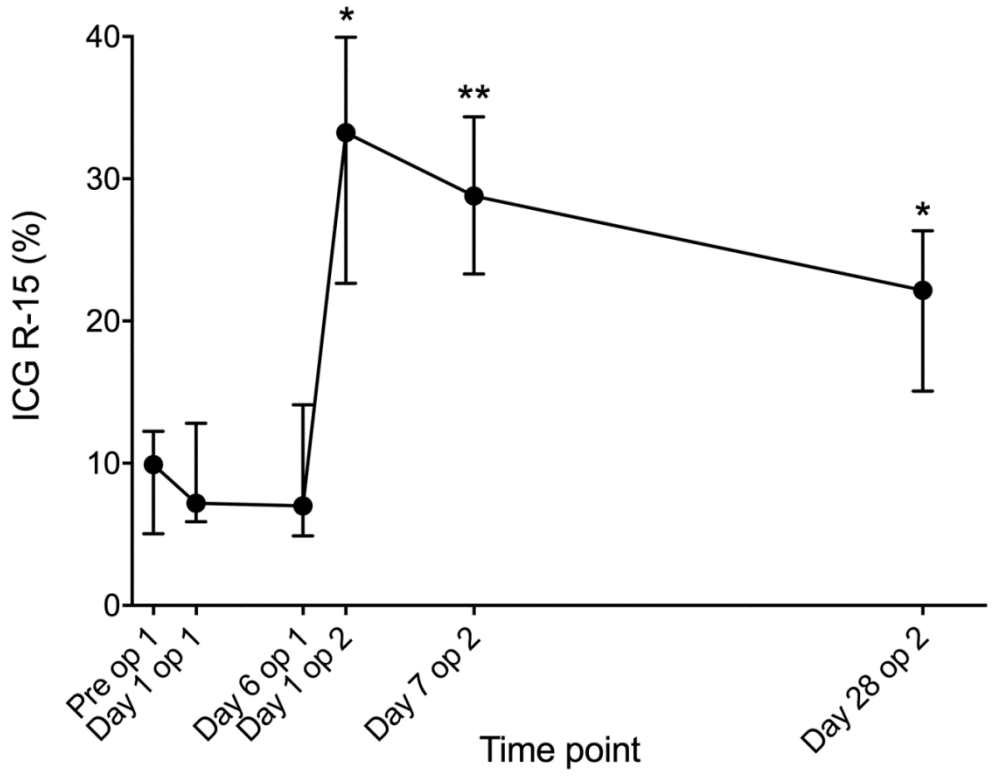
5.3 AIM III: FLR VOLUME VS FUNCTION AFTER ALPPS

In **Paper III** nine patients with CRLM were included into the study. All patients received neoadjuvant chemotherapy comprising a median of six cycles (range 4-14). In five patients the liver metastases were detected synchronously to the primary tumour and two patients had tumour manifestation in the FLR in addition to the right liver lobe and segment IV. Six patients had previous failed PVO (PVE 3; PVL 2; PVL and PVE 1) prior to inclusion, meaning that PVO did not induce sufficient growth of the FLR with a FLR/BW still below 0.5% after evaluation of the PVO effect. The stage 2 operation was performed in all patients seven days after the stage 1 operation.

All patients completed the stage 2 operation with removal of segments IV-VIII. Pathological examination showed radical resection (R0) in seven patients while two patients had tumour cells within 1 mm of the resection line (R1). One patient with bile leakage after stage 2 required endoscopic stenting under general anaesthesia thus denoted as a grade 3b-complication. Three patients had pleural effusions requiring drainage under local anaesthetic (grade 3a-complication). No patient developed severe posthepatectomy liver failure (PHLF) and there was no 90-day mortality.

The median ICG-R15 on the day before stage 1 operation was 9.9% (range 1.2-20.7) and on day 6 after stage 1 7.0% (4.2-19.5). There was a significant rise in ICG-R15 directly after stage 2 (33.3%, 8.5-43.2, p=0.012) that was maintained on day 7 after stage 2 (28.8%, 19.2-38.9) and on day 28 after stage 2 (22.2%, 10.3-37.5).

Figure 5. *Dynamic evaluation of ICG clearance in ALPPS.*



*Indocyanine green retention at 15 minutes (ICG-R15%) at six time points before and after both stages of the ALPPS procedure. *p<0.05, **p<0.01.*

The median preoperative FLR-volume was 300 ml (range 260-433) translating into a median sFLR of 19.5% (16.1-25.8). On day 6 after stage 1 and days 7 and 28 after stage 2 the FLR-volume had increased to 557, 700 and 793 ml respectively (p=0.008, 0.011 and 0.008), translating into a median sFLR of 33.1, 40.6 and 48.0%. The median FLR-volume percentage increase on day 6 after stage 1, day 7 and day 28 after stage 2 were 56.7% (range 32.3-110.4), 114.7% (48.8-174.3) and 132% (90-218.3) using the preoperative FLR-volume and TELV as references. Median FLR/BW before stage 1 was 0.41% (range 0.35-0.49) and increased to 0.71% (0.54-0.90, p=0.008) prior to stage 2. The median KGR of the FLR-volume was 9.4%/day (range 5.4-18.4) during the six days between stage 1 and 2, 3.8%/day (-0.3-8.2) during the first seven days after stage 2 and 0.5%/day (0.1-4.2) from day 8 to 28 after stage 2 (volumetric data of the FLR are summarized in **Figure 6a**).

The median preoperative FLR-function was 1.8%/min/m^2 (range 1.4-2.9), translating into a median FLR-function/TL-function share of 25.3% (19.3-33.1). On day 6 after stage 1 and days 7 and 28 after stage 2 the median FLR-function had increased to 2.6, 3.4 and 4.1%/min/m^2 respectively (p=0.051, 0.036 and 0.011), resulting in FLR-function/TL-function shares of 33.9, 43.7 and 55.5%. Using the preoperative FLR-function and TL-function share as reference the median FLR-function increase on day 6 after stage 1, day 7 and day 28 after stage 2 were 28.2% (range -35.7-83.8), 66.4% (0.7-147.5) and 92.2% (47.3-191.5)

respectively. It was notable that the median FLR uptake rate per volume unit was decreased significantly on day six after stage 1 (8.5%/min/l) compared to preoperatively (11.8%/min/l, p=0.028), and did not surpass the preoperative values on day 7 (9.0%/min/l) or on 28 after stage 2 (10.1%/min/l). The median KGR of the FLR-function was 4.7%/day (range -6-14) during the six days between stage 1 and 2. During the seven days following stage 2 it was 4.9%/day (range -2-24.9) and from day 8 to 28 after stage 2 it decreased to 1.4%/day (-0.3-2.2) (functional data of the FLR are summarized in **Figure 6b**).

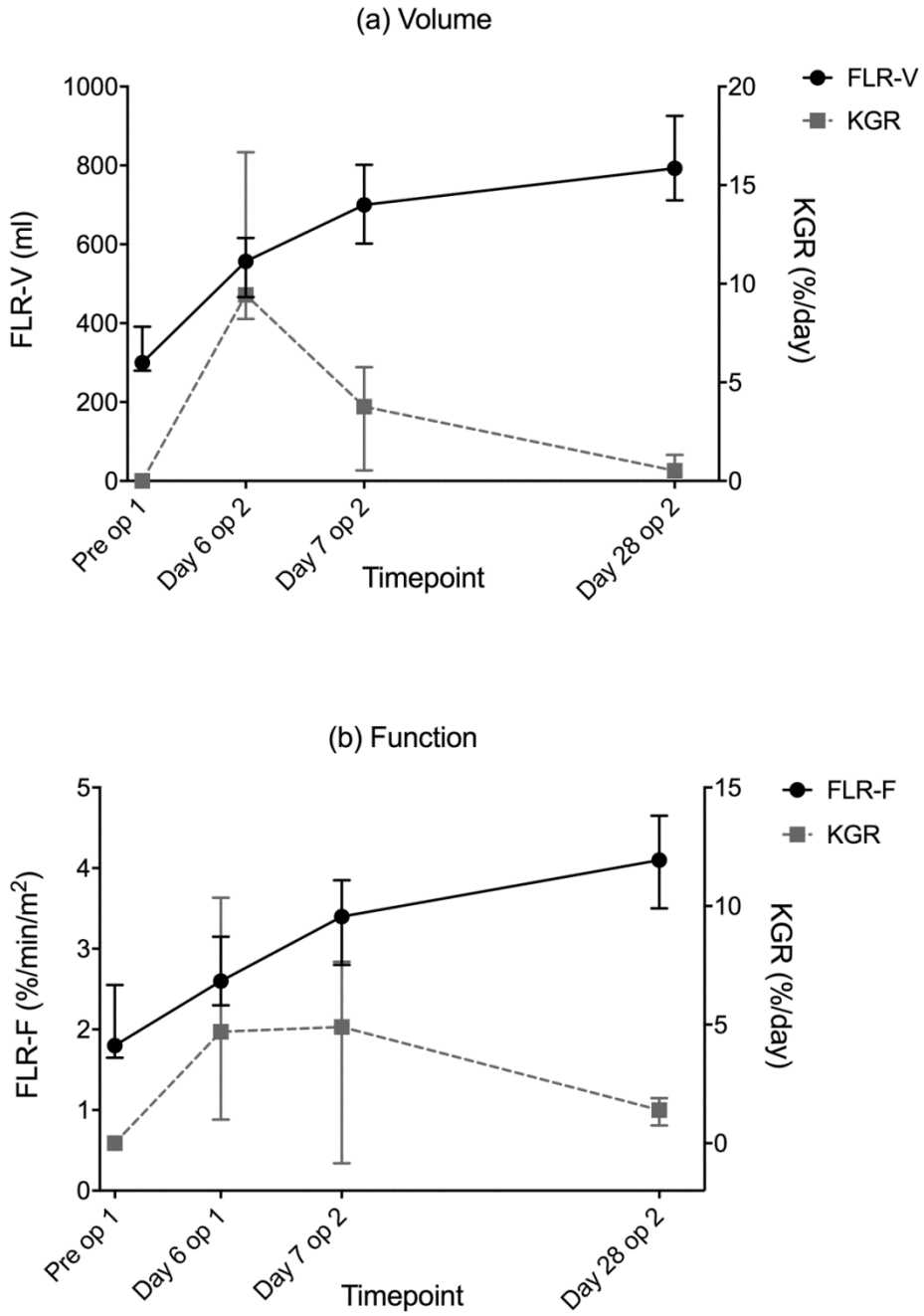
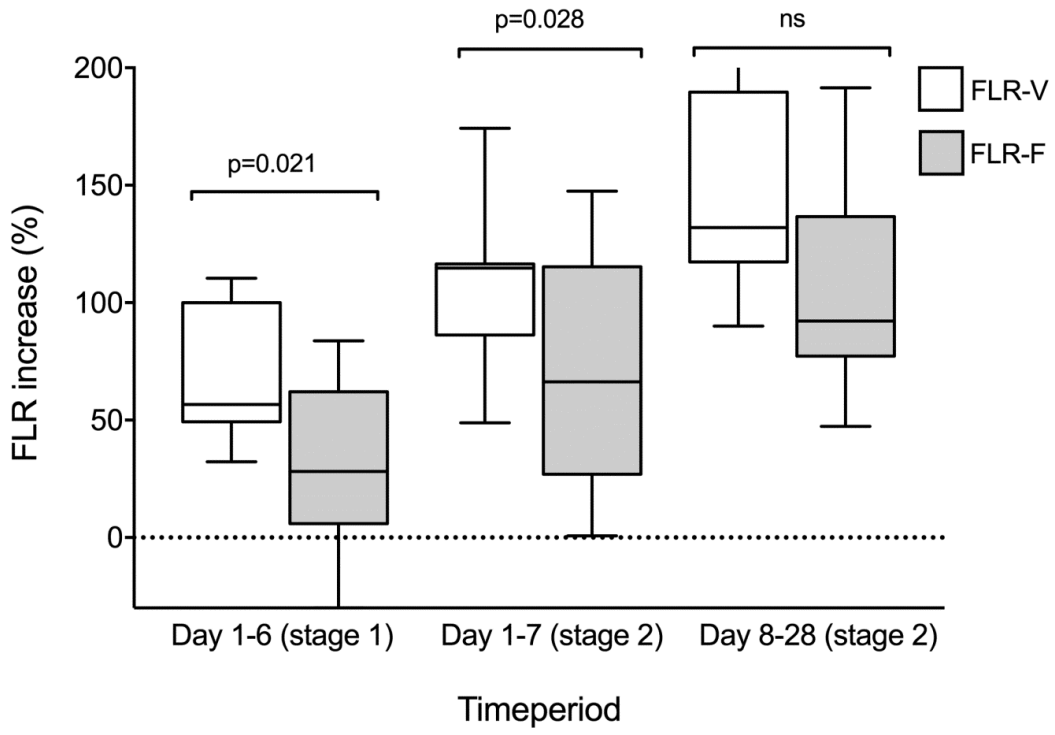


Figure 6. *(A) FLR-volume (ml) compared to kinetic growth rate (KGR) of volume increase (%/day) at the four time points; pre stage 1, day 6 after stage 1, day 7 and 28 after stage 2. (B) FLR-function (%/min/m^2) compared to KGR of function increase (%/day) at the same time points.*

The preoperative sFLR of 19.5% underestimated the preoperative FLR-function/TL-function share of 25.3% (p=0.011). The median increase in volume exceeded the increase in function at day six after stage 1 (FLR-volume increase 56.7% versus FLR-function increase 28.2%, p=0.021). The increase in volume still exceeded increase in function the first seven days after stage 2 (FLR-volume increase 114.7% versus FLR-function increase 66.4%, p=0.028) but with greater functional growth rate after stage 2 resulted in comparable levels on day 28 (FLR-volume increase 132.0% versus FLR-function increase 92.2%, p=0.11), with the preoperative FLR-volume and FLR-function as reference.

Figure 7. *Volume versus Function.*



Comparison of percentage increase in FLR-volume (FLR-V) and FLR-function (FLR-F) day 6 after stage 1 and days 7 and 28 after stage 2, with preoperative FLR-V and FLR-F as reference.

The ALPPS procedure has been suggested as an alternative to PVO for inducing hypertrophy of the FLR [12]. Proponents claim a more rapid increase in FLR as compared to the traditional methods for FLR manipulation [111]. Resection rates for patients with CRLM after ALPPS of 97-100% have been reported, compared to around 70-80% after PVO [111]. Tumour recurrence rates at 1-year follow-up for ALPPS and PVO were comparable [97]. Sceptics have raised concern that the extreme increase in FLR size is not necessarily the

result of true hypertrophy [100]. Furthermore the relationship between hypertrophy and increased function has been questioned [134, 135].

In the present study a 56.7% median increase in FLR-volume was paired with a 28.2% FLR-function increase day 6 after stage 1, or in other words the functional increase represented only 50% of the increase in volume. This gives some legitimacy to fears that the fast initial growth in volume after ALPPS does not translate into an equivalent increase in function. This may, in part, explain the observation that extreme hypertrophy does not necessarily ensure a sufficient FLR and safe postoperative course [107].

In a letter to the editor, Lau et al presented a case report implying the use of repeated ICG measurement for resectability decision-making in ALPPS [136]. In the present study ICG values did not increase directly after stage 1 or prior to stage 2. The pronounced increase in ICG seen from day 1 after stage 2 did not translate into liver failure, and might simply be a result of increased blood flow to the liver remnant after resecting the DPL. Whether a sharp increase in ICG prior to stage 2 could indicate a need to postpone stage 2 operation remains to be investigated in larger cohorts of patients.

5.4 AIM IV: CHARACTERIZATION OF GROWTH FACTORS IN ALPPS

In **Paper IV** ten patients with CRLM were included into the study. All patients received neoadjuvant chemotherapy containing a median of six cycles. Seven patients had failed PVO (PVE 4; PVL 2; PVL and PVE 1) before inclusion, meaning that PVO did not induce sufficient growth of the FLR with a FLR/BW still below 0.5% after evaluation of the PVO effect.

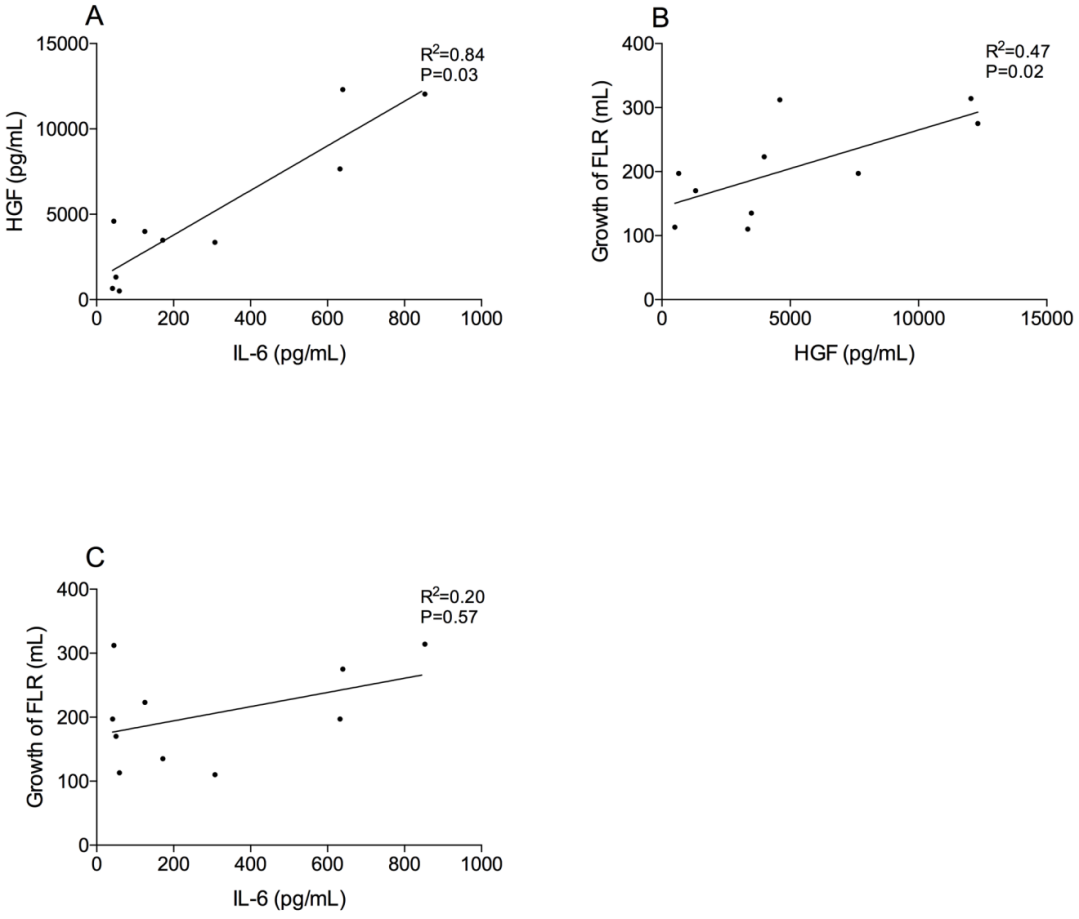
The preoperative FLR was 342 ml (range 260-433) translating into a FLR/BW of 0.41% and a sFLR of 19.7%. On day 6 after stage 1 the FLR had increased significantly to 562 ml ($p<0.001$), resulting in a FLR/BW of 0.64% and a sFLR of 30.6%. The median FLR percentage increase on day 6 after stage 1 was 55.7% (32-120). KGR of the FLR was 9.3%/day (5.4-20) during the six days between stage 1 and 2. The hypertrophy of the liver remnant slowed down after stage 2 but the increase was still significant at day 7 and 28 ($p=0.01$ and 0.004 respectively). The liver remnant accounted for 41.7% of the TELV seven days after stage 2 and 48% 28 days after stage 2; meaning that the liver remnant four weeks after completed ALPPS still did not reach half the preoperative TELV.

Plasma levels of IL-6 increased at D1 OP1 ($p=0.004$) compared to baseline. The concentration then declined but was still higher compared to baseline at D-1 OP2 ($p=0.002$), D1 OP2 ($p=0.004$) and D7 OP2 ($p=0.008$). Plasma EGF decreased compared to baseline at D1 OP1 ($p=0.004$) and was lower also at D-1 OP2 ($p=0.01$), D1 OP2 ($p=0.004$) and D7 OP2 ($p=0.015$). TNF-alpha declined at D1 OP1 compared to baseline ($p=0.02$). There was no difference compared to baseline at D-1 OP2 ($p=0.65$), but was significantly lower at D-1 OP2 ($p=0.045$) and at D7 OP2 ($p=0.04$) compared to baseline. VEGF was lower at D1 OP1

(p=0.01) compared to baseline. Because of inter-individual variations in the time-point for the peak concentration of the evaluated factors in combination with a limited sample size, the differences between baseline concentrations and peak concentrations of each factor were assessed. There was a significant change in plasma levels between baseline and peak for IL-6, HGF and VEGF (p=0.02, p=0.048 and p=0.048, respectively).

To evaluate the relation between plasma markers for liver regeneration and growth of FLR, regression analysis was performed. There was a strong correlation between peak plasma concentrations of IL-6 and HGF (R^2=0.83, p=0.03). Furthermore, HGF correlated to the growth of FLR at D-1 OP2 (R^2=0.47, p=0.02), though the growth did not correlate to IL-6 (R^2=0.20, p=0.57).

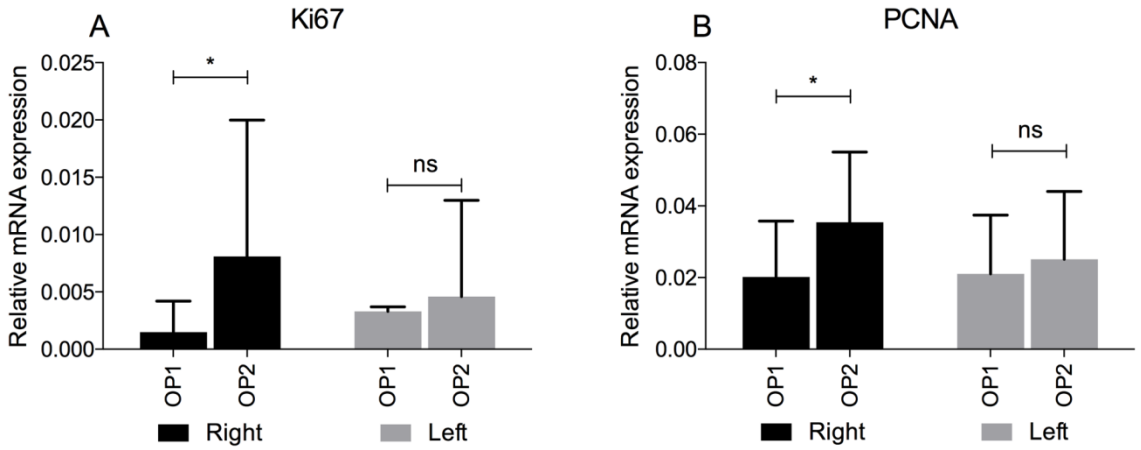
Figure 8. *Peak levels of HGF correlates to peak levels of IL-6 and growth of FLR.*



Correlation between (A) peak levels of HGF and IL-6, (B) peak levels of HGF and growth of FLR between baseline and D-1 OP2, (C) peak levels of IL-6 and growth of FLR between baseline and D-1 OP2 (n=10).

Expression of Ki67 and PCNA (proliferating cell nuclear antigen) increased in both lobes at stage 2 compared to stage 1. The increase was more prominent in the right lobe compared to the left lobe (right lobe; p=0.03 and p=0.05, left lobe; p=0.12 and p=0.69).

Figure 9 *Ki67 and PCNA mRNA expression in ALPPS.*



Ki67 (A) and PCNA (B) mRNA expression increases significantly in the right lobe, but not the left, between stage 1 and 2.

IL-6 has been suggested to stimulate the production of HGF in liver regeneration [137, 138] which is consistent with the results of the present study, in that high levels of IL-6 directly after stage 1 correlated with high HGF levels. Furthermore, high levels of HGF correlated with a greater growth of the FLR. The rapid increase in IL-6 is similar to that described by Sasturkar et al [22] but in that study in healthy donors peak levels of HGF were detected later than in the present study. In the study by Matsumoto et al [21], also in healthy donors, HGF peaked early after partial hepatectomy as reported in the present study. In addition, a late expression of VEGF was seen, similar to what is shown in this study.

Several attempts to study proliferation in ALPPS [27, 139] have shown increased proliferative markers in the FLR. One advantage with the current study is that the deportalized liver was used as internal control in that bilobar biopsies at both operations were compared in all patients. Although a trend towards elevation in mRNA expression of both Ki-67 and PCNA was seen in the FLR between both stages, there was a greater increase in the deportalized lobe. This appears to be a paradox, but it might represent a response to apoptosis in the hypoxic liver. Schadde et al recently described that tissue hypoxia in the FLR after ALPPS lead to a higher degree of regeneration [140].

Another interesting finding is that the initial fast and powerful hypertrophy after stage 1, slowed down significantly after stage 2. In fact, 28 days after stage 2 the liver remnant still

did not reach half the initial size of the liver and with a much lower KGR than in other settings [141].

The present study is the first to describe serial measurements of multiple growth factors in plasma in humans subjected to ALPPS and correlate these levels with a detailed volumetric study after both stages of the procedure. In addition, this study present an analysis of mRNA expression of proliferative and apoptotic markers in both liver lobes at both stages of ALPPS.

5.5 GENERAL DISCUSSION AND CLINICAL IMPLICATIONS

A general limitation for all the papers in this thesis is the limited number of included patients in each study. This is a common problem for most studies in the field of PVO and ALPPS. Still, for several results in this thesis, other recent studies report similar conclusions. Until more solid evidence is presented, interpretation of these results always has to be undertaken with this limitation in mind. Another concern regards the selection of patients for the studies. In particular, when analysing only rescue ALPPS patients there is an obvious built-in selection bias. Patients previously subjected to PVO that either have a satisfactory growth of the FLR or experience tumour progression after PVO excluding them from curative surgery, are not analysed in the mentioned setup.

Recent attempts to compare early survival after ALPPS with two-stage hepatectomy [142] or even palliative chemotherapy [143] for patients with advanced liver metastatic disease from colorectal cancer indicate poor results for ALPPS, not even being superior to palliative chemotherapy. This is of course concerning, but several objections could be raised on the methodology in the studies, making generalisation of the results premature. Despite that, an important conclusion is that patient selection in ALPPS is pivotal and that further studies are warranted.

If the results from **Paper I** could be confirmed in larger studies it would have the potential to change our current approach when planning surgical strategy for patients with colorectal liver metastases in the FLR. Instead of TSH these patients could be managed with percutaneous PVE shortly after ended chemotherapy and a subsequent one-stage operation with complete resection of all tumours.

In **Paper II** we confirmed the results from previous reports, that the pronounced growth of FLR associated with ALPPS, is maintained after rescue ALPPS despite of previous PVO in this setting. There are also indications that both volume and function increase of the FLR after PVE is faster than currently estimated [69, 144]. If this is confirmed it could change the way we use PVE and also lay the foundations for a new strategy in dealing with a small FLR. This potential strategy would comprise preference to use PVE in small FLR with earlier evaluation of the PVE effect, and then rescue ALPPS taking advantage of the second growth of the FLR. Of course this treatment strategy would have to be confirmed scientifically before a broader implementation can be recommended.

The need for an accurate tool for functional assessment of the FLR, in particular in ALPPS, is gaining attention in the hepatobiliary community. The described morbidity and mortality associated with ALPPS (mainly due to PHLF despite adequate FLR volumes prior to stage 2), together with the results from **Paper III**, have altered the management of ALPPS patients at Karolinska University Hospital. Our current strategy is to supplement the volume measurement of the FLR (based on CE-CT before stage 1 and seven days after stage 1, i.e. the day before stage 2) with HBS and ICG clearance test before both stages of ALPPS. If we observe a satisfactory increase in FLR-volume before stage 2, but the ICG-value are significantly increased or HBS indicate a poor FLR-function, the stage 2 procedure is postponed.

Paper IV lack obvious clinical implications because of its descriptive nature, but could engage in more translational studies for enhanced understanding of liver regeneration in general.

6 CONCLUSIONS

This thesis provides results to support the following conclusions:

The rate of progression of CRLM after PVE and pre-procedural chemotherapy is lower than previously reported if the time between the end of chemotherapy and PVE is short.

The risk of tumour progression is low both in the embolized and non-embolized liver.

The powerful growth of the FLR associated with ALPPS seems to be maintained in patients with CRLM treated with neoadjuvant chemotherapy and previously failed PVO.

In patients with CRLM treated with neoadjuvant chemotherapy and previously failed PVO, ALPPS can be performed with low morbidity and high resection rate.

During the inter-stage period of ALPPS the high volume increase is not paralleled by a corresponding functional increase.

The demonstrated discrepancy between volume and function in the FLR prior to stage 2 warrants additional functional assessment before proceeding to stage 2.

IL-6, HGF and TNF-α seem to be early mediators of hypertrophy after stage 1 in the ALPPS procedure, whereas EGF and VEGF seem to increase later.

The levels of HGF correlates significantly with the degree of growth of the FLR in patients subjected to ALPPS.

The levels of IL-6 correlates significantly with the levels of HGF in patients subjected to ALPPS.

7 FUTURE RESEARCH

There is a remaining need to investigate the issues in this thesis further.

The volumetric assessment of the liver in connection with both portal vein occlusion and liver resection are being explored in several projects that we have initiated, both locally and together with other hepatobiliary centres in Sweden.

Recently the first randomized controlled study comparing portal vein occlusion and ALPPS completed the enrolment of patients (actually the first RCT in ALPPS). It is a Nordic multi-centre study and we are one of the contributing centres. Data analysis is being performed and the presentation of the results from the study will be of great importance in regard to the future use of these procedures.

The network of the participants from the first international expert meeting in ALPPS held in Hamburg in 2015 created a platform for research collaborations. Recently an international multi-centre study pooling all hepatobiliary scintigraphies in ALPPS from four participating centres (including Stockholm) was performed. An additional initiative was recently conveyed by the coordinator of the International ALPPS Registry with the aim of collecting all patients subjected to ALPPS with HBS for evaluation of FLR function in a world-wide setting. Hopefully this can lead to increased knowledge of this promising method for assessment of the functional FLR and potentially contribute to a decreased occurrence of posthepatectomy liver failure after ALPPS.

Since portal vein occlusion and ALPPS are used to avoid posthepatectomy liver failure further studies, taking advantage of the interface between these research fields, will be received with great interest.

The ALPPS procedure has generated significant interest as a promising model to study liver regeneration in humans. This creates a favourable foundation for translational studies that could contribute to a deepened understanding of the complex mechanisms behind liver regeneration.

In summary, for me the work with this thesis has created a base for future research with the translational, national and international projects being especially rewarding.

8 POPULÄRVETENSKAPLIG SAMMANFATTNING

Vid tumörer i levern kan kirurgisk behandling erbjuda bot för patienten. En förutsättning för att leverkirurgi med botande syfte ska kunna utföras är att patienten har tillräckligt stor kvarvarande lever efter operationen. Om den kvarvarande levern efter operation är för liten löper patienten stor risk att utveckla postoperativ leversvikt, vilket är den allvarligaste komplikationen till leverkirurgi. Därför använder man röntgen för att mäta volymen på den friska delen av levern innan man opererar patienten. Ett problem med denna volyms-baserade beslutsgrund inför leverkirurgi är att den inte tar tillräcklig hänsyn till kvalitet och funktion i den delen av levern som patienten har kvar efter operation. Det gör att vissa patienter utvecklar postoperativ leversvikt trots att volymen på den kvarvarande levern ansågs vara tillräcklig innan kirurgi. Ett annat problem är när volymen på den friska, icke tumörbärande delen av levern är för liten. Dessa patienter har tidigare blivit exkluderade från potentiellt botande kirurgi på grund av den stora risken för postoperativ leversvikt.

En metod som kan användas för att skapa tillväxt i den friska delen av levern är selektiv portavensavstängning av portavenen till den sjuka delen av levern. Portavenen är det blodkärl som transporterar den näring som tas upp från tarmen till levern. Principen bakom portavensavstängning är att styra det näringsrika portablodet till den friska delen av levern för att få denna del att bli större innan leverkirurgi. Portavensavstängning kan antingen utföras med hjälp av röntgen - det kallas då portavensembolisering - eller med en kirurgisk delning av portavenen till den sjuka delen av levern. Om denna metod är framgångsrik skapar den tillräckligt stor tillväxt av den friska delen av levern så att levertumörerna några veckor senare kan opereras bort utan stor risk för postoperativ leversvikt. Även om en stor del av patienterna når målet med denna metod är det cirka 20-30 % som antingen inte får tillräckligt stor tillväxt eller att tumörerna växer för mycket i väntan på effekten av portavensavstängningen. Dessa patienter har tidigare inte kunnat erbjudas botande behandling.

En ny kirurgisk metod som kallas ALPPS (associating liver partition and portal vein ligation for staged hepatectomy) har sedan den presenterades 2012 visat sig vara mycket effektiv när det gäller att skapa snabb och kraftfull tillväxt av den friska delen av levern. Det är en tvåstegsoperation där man vid första operationen delar portavenen till den tumörbärande delen av levern (precis som vid kirurgisk portavensdelning) och samtidigt klyver man levern mellan den sjuka och friska delen. Efter att man utfört en ny röntgen cirka sju dagar efter första operationen och sett att den friska levern växt tillräckligt mycket, genomförs den andra operationen där man avlägsnar den tumörbärande delen av levern. I **Figure 1** på sidan 7 har metoden illustrerats. De första rapporterna om denna metod visade att den friska levern växte så pass mycket att nästan samtliga patienter (97-100 %) kunde genomgå även den andra operationen och bli av med sina levertumörer. Dock höjdes ett

varnande finger för att metoden var förknippad med större komplikationsrisk och även högre dödlighet till följd av operationen, jämfört med annan leverkirurgi.

Det aktuella avhandlingsarbetet studerade ovanstående tekniker i fyra delarbeten.

I delarbete I inkluderades 34 patienter med tjock- och ändtarmscancer som utvecklat dottertumörer i levern och behandlats vid Skånes Universitetssjukhus och Karolinska Universitetssjukhuset. Samtliga patienter i studien hade för liten kvarvarande lever för att kunna opereras i levern direkt och behandlades därför med portavensemboliserings. Innan portavensemboliseringen hade patienterna fått förbehandling med cellgifter. Syftet med studien var att undersöka om dottermetastaserna i levern växte i väntan på effekten av portavensemboliseringen. Det viktigaste resultatet av studien var att om tiden mellan avslutad cellgiftsbehandling och portavensemboliserings hålls kort, så är risken för oönskad tillväxt av dottertumörerna låg.

I delarbete II undersöktes om ALPPS kan användas efter misslyckad portavensavstängning som ett sista försök att bota patienten. Studien inkluderade elva patienter med tjock- och ändtarmscancer som utvecklat dottertumörer i levern och genomgått portavensavstängning utan tillräcklig tillväxt av den friska levern. För det första sågs en liknande tillväxt som vid de första rapporterna om ALPPS, trots att dessa patienter tidigare genomgått portaven-savstängning. För det andra kunde samtliga patienter genomgå även den andra operationen och bli av med sina levertumörer utan hög komplikationsfrekvens och helt utan dödlighet till följd av ingreppet.

I delarbete III studerades leverns tillväxt och funktion i samband med ALPPS med hjälp av flera olika metoder (skiktröntgen, gammakamera, indocyaningrönt leverfunktionstest och blodprover). Studien inkluderade nio patienter med tjock- och ändtarmscancer som utvecklat dottertumörer i levern och som opererades med ALPPS. Resultaten visar att den kraftiga volymstillväxten av den friska levern efter första operationen i ALPPS inte åtföljs av en lika stor funktionell ökning. Detta skulle kunna förklara den höga frekvens leversvikt som beskrivits efter andra operationen i ALPPS, trots att adekvat volymstillväxt uppmätts.

I delarbete IV studerades nivåerna av tillväxtfaktorer med betydelse för levertillväxt i vävnad hos tio patienter som genomgått ALPPS. Upprepad blodprovstagning utfördes före och efter båda operationerna i ALPPS. Vidare togs vävnadsprov från levern vid båda operationerna. Dessa vävnadsprover analyserades sedan för ett antal tillväxtfaktorer och jämfördes med tillväxtgraden i den friska levern. Studien har för första gången gett oss en bild av uttrycket av dessa tillväxtfaktorer efter utförd ALPPS hos människa.

Sammanfattningsvis har studierna i denna avhandling bidragit till att öka förståelsen för vissa aspekter vid portavensavstängning och ALPPS.

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10 REFERENCES

1. Poon, R.T., et al., *Improving Perioperative Outcome Expands the Role of Hepatectomy in Management of Benign and Malignant Hepatobiliary Diseases.* Transactions of the ... Meeting of the American Surgical Association, 2004. **CXXII**(&NA;): p. 296-308.
2. Jarnagin, W.R., et al., *Improvement in perioperative outcome after hepatic resection: analysis of 1,803 consecutive cases over the past decade.* Ann Surg, 2002. **236**(4): p. 397-406; discussion 406-7.
3. Pawlik, T.M., R.D. Schulick, and M.A. Choti, *Expanding criteria for resectability of colorectal liver metastases.* Oncologist, 2008. **13**(1): p. 51-64.
4. Ekberg, H., et al., *Determinants of survival in liver resection for colorectal secondaries.* Br J Surg, 1986. **73**(9): p. 727-31.
5. Balzan, S., et al., *The "50-50 Criteria" on Postoperative Day 5: an accurate predictor of liver failure and death after hepatectomy.* Annals of Surgery, 2005. **242**(6): p. 824-829.
6. Ferrero, A., et al., *Postoperative liver dysfunction and future remnant liver: where is the limit? Results of a prospective study.* World J Surg, 2007. **31**(8): p. 1643-51.
7. Garcea, G. and G.J. Maddern, *Liver failure after major hepatic resection.* J Hepatobiliary Pancreat Surg, 2009. **16**(2): p. 145-55.
8. Rahbari, N.N., et al., *Posthepatectomy liver failure: a definition and grading by the International Study Group of Liver Surgery (ISGLS).* Surgery, 2011. **149**(5): p. 713-24.
9. Shoup, M., et al., *Volumetric analysis predicts hepatic dysfunction in patients undergoing major liver resection.* J Gastrointest Surg, 2003. **7**(3): p. 325-30.
10. Makuuchi, M., et al., *Preoperative portal embolization to increase safety of major hepatectomy for hilar bile duct carcinoma: a preliminary report.* Surgery, 1990. **107**(5): p. 521-7.
11. Jaeck, D., et al., *A Two-Stage Hepatectomy Procedure Combined With Portal Vein Embolization to Achieve Curative Resection for Initially Unresectable Multiple and Bilobar Colorectal Liver Metastases.* Annals of Surgery, 2004. **240**(6): p. 1037-1051.
12. Schnitzbauer, A.A., et al., *Right portal vein ligation combined with in situ splitting induces rapid left lateral liver lobe hypertrophy enabling 2-staged extended right hepatic resection in small-for-size settings.* Ann Surg, 2012. **255**(3): p. 405-14.
13. Guglielmi, A., et al., *How much remnant is enough in liver resection?* Dig Surg, 2012. **29**(1): p. 6-17.
14. Court, F.G., et al., *The mystery of liver regeneration.* Br J Surg, 2002. **89**(9): p. 1089-95.
15. Fausto, N., J.S. Campbell, and K.J. Riehle, *Liver regeneration.* Hepatology, 2006. **43**(2 Suppl 1): p. S45-53.

16. Hayashi, H., et al., *Increase in the serum bile acid level predicts the effective hypertrophy of the nonembolized hepatic lobe after right portal vein embolization.* World J Surg, 2009. **33**(9): p. 1933-40.

17. Michalopoulos, G.K., *Liver regeneration after partial hepatectomy: critical analysis of mechanistic dilemmas.* Am J Pathol, 2010. **176**(1): p. 2-13.

18. Tashiro, S., *Mechanism of liver regeneration after liver resection and portal vein embolization (ligation) is different?* J Hepatobiliary Pancreat Surg, 2009. **16**(3): p. 292-9.

19. Michalopoulos, G.K., *Liver regeneration.* J Cell Physiol, 2007. **213**(2): p. 286-300.

20. Michalopoulos, G.K. and M.C. DeFrances, *Liver regeneration.* Science, 1997. **276**(5309): p. 60-6.

21. Matsumoto, K., et al., *Serial changes of serum growth factor levels and liver regeneration after partial hepatectomy in healthy humans.* Int J Mol Sci, 2013. **14**(10): p. 20877-89.

22. Sasturkar, S.V., et al., *Serial changes of cytokines and growth factors in peripheral circulation after right lobe donor hepatectomy.* Liver Transpl, 2016. **22**(3): p. 344-51.

23. Marcos, A., et al., *Liver regeneration and function in donor and recipient after right lobe adult to adult living donor liver transplantation.* Transplantation, 2000. **69**(7): p. 1375-9.

24. de Jong, K.P., et al., *Serum response of hepatocyte growth factor, insulin-like growth factor-I, interleukin-6, and acute phase proteins in patients with colorectal liver metastases treated with partial hepatectomy or cryosurgery.* J Hepatol, 2001. **34**(3): p. 422-7.

25. Schlegel, A., et al., *ALPPS: from human to mice highlighting accelerated and novel mechanisms of liver regeneration.* Ann Surg, 2014. **260**(5): p. 839-46; discussion 846-7.

26. Garcia-Perez, R., et al., *Associated Liver Partition and Portal Vein Ligation (ALPPS) vs Selective Portal Vein Ligation (PVL) for Staged Hepatectomy in a Rat Model. Similar Regenerative Response?* PLoS One, 2015. **10**(12): p. e0144096.

27. de Santibanes, M., et al., *Biological Substrate of the Rapid Volumetric Changes Observed in the Human Liver During the Associating Liver Partition and Portal Vein Ligation for Staged Hepatectomy Approach.* J Gastrointest Surg, 2016. **20**(3): p. 546-53.

28. Matsuo, K., et al., *Histologic features after surgery associating liver partition and portal vein ligation for staged hepatectomy versus those after hepatectomy with portal vein embolization.* Surgery, 2016. **159**(5): p. 1289-98.

29. Schreckenbach, T., et al., *Posthepatectomy liver failure.* Dig Surg, 2012. **29**(1): p. 79-85.

30. van den Broek, M.A., et al., *Liver failure after partial hepatic resection: definition, pathophysiology, risk factors and treatment.* Liver Int, 2008. **28**(6): p. 767-80.

31. Lafaro, K., et al., *Defining Post Hepatectomy Liver Insufficiency: Where do We stand?* J Gastrointest Surg, 2015. **19**(11): p. 2079-92.

32. Mullen, J.T., et al., *Hepatic insufficiency and mortality in 1,059 noncirrhotic patients undergoing major hepatectomy.* J Am Coll Surg, 2007. **204**(5): p. 854-62; discussion 862-4.

33. Little, S.A., et al., *Diabetes is associated with increased perioperative mortality but equivalent long-term outcome after hepatic resection for colorectal cancer.* J Gastrointest Surg, 2002. **6**(1): p. 88-94.

34. Balzan, S., et al., *Safety of liver resections in obese and overweight patients.* World J Surg, 2010. **34**(12): p. 2960-8.

35. Robinson, S.M., et al., *Chemotherapy-associated liver injury in patients with colorectal liver metastases: a systematic review and meta-analysis.* Ann Surg Oncol, 2012. **19**(13): p. 4287-99.

36. Vauthey, J.N., et al., *Chemotherapy regimen predicts steatohepatitis and an increase in 90-day mortality after surgery for hepatic colorectal metastases.* J Clin Oncol, 2006. **24**(13): p. 2065-72.

37. Bismuth, H., et al., *Liver resections in cirrhotic patients: a Western experience.* World J Surg, 1986. **10**(2): p. 311-7.

38. Poon, R.T., et al., *Extended hepatic resection for hepatocellular carcinoma in patients with cirrhosis: is it justified?* Ann Surg, 2002. **236**(5): p. 602-11.

39. Cherqui, D., et al., *Major liver resection for carcinoma in jaundiced patients without preoperative biliary drainage.* Arch Surg, 2000. **135**(3): p. 302-8.

40. Kennedy, T.J., et al., *Role of preoperative biliary drainage of liver remnant prior to extended liver resection for hilar cholangiocarcinoma.* HPB (Oxford), 2009. **11**(5): p. 445-51.

41. van Mierlo, K.M., et al., *Liver resection for cancer: New developments in prediction, prevention and management of postresectional liver failure.* J Hepatol, 2016. **65**(6): p. 1217-1231.

42. Abdalla, E.K., et al., *Total and segmental liver volume variations: implications for liver surgery.* Surgery, 2004. **135**(4): p. 404-10.

43. Kubota, K., et al., *Measurement of liver volume and hepatic functional reserve as a guide to decision-making in resectional surgery for hepatic tumors.* Hepatology, 1997. **26**(5): p. 1176-81.

44. Shirabe, K., et al., *Postoperative liver failure after major hepatic resection for hepatocellular carcinoma in the modern era with special reference to remnant liver volume.* J Am Coll Surg, 1999. **188**(3): p. 304-9.

45. Emond, J.C., et al., *Functional analysis of grafts from living donors. Implications for the treatment of older recipients.* Ann Surg, 1996. **224**(4): p. 544-52; discussion 552-4.

46. Kiuchi, T., et al., *Impact of graft size mismatching on graft prognosis in liver transplantation from living donors.* Transplantation, 1999. **67**(2): p. 321-7.

47. Truant, S., et al., *Remnant liver volume to body weight ratio > or =0.5%: A new cut-off to estimate postoperative risks after extended resection in noncirrhotic liver.* J Am Coll Surg, 2007. **204**(1): p. 22-33.

48. Urata, K., et al., *Calculation of child and adult standard liver volume for liver transplantation.* Hepatology, 1995. **21**(5): p. 1317-21.

49. Ribero, D., Y.S. Chun, and J.N. Vauthey, *Standardized liver volumetry for portal vein embolization.* Semin Intervent Radiol, 2008. **25**(2): p. 104-9.

50. Vauthey, J.N., et al., *Standardized measurement of the future liver remnant prior to extended liver resection: methodology and clinical associations.* Surgery, 2000. **127**(5): p. 512-9.

51. Chun, Y.S., et al., *Comparison of two methods of future liver remnant volume measurement.* J Gastrointest Surg, 2008. **12**(1): p. 123-8.

52. Martel, G., et al., *Comparison of techniques for volumetric analysis of the future liver remnant: implications for major hepatic resections.* HPB (Oxford), 2015.

53. Ribero, D., et al., *Measured versus estimated total liver volume to preoperatively assess the adequacy of the future liver remnant: which method should we use?* Ann Surg, 2013. **258**(5): p. 801-6; discussion 806-7.

54. Clavien, P.A., et al., *Strategies for safer liver surgery and partial liver transplantation.* N Engl J Med, 2007. **356**(15): p. 1545-59.

55. Abdalla, E.K. and J.N. Vauthey, *Improved survival after resection of colorectal liver metastases.* J Clin Oncol, 2006. **24**(17): p. 2679; author reply 2680-1.

56. Kishi, Y., et al., *Three hundred and one consecutive extended right hepatectomies: evaluation of outcome based on systematic liver volumetry.* Ann Surg, 2009. **250**(4): p. 540-8.

57. Imamura, H., et al., *Assessment of hepatic reserve for indication of hepatic resection: decision tree incorporating indocyanine green test.* J Hepatobiliary Pancreat Surg, 2005. **12**(1): p. 16-22.

58. Suda, K., et al., *Risk factors of liver dysfunction after extended hepatic resection in biliary tract malignancies.* Am J Surg, 2009. **197**(6): p. 752-8.

59. Takahashi, T., et al., *Safe and permissible limits of hepatectomy in obstructive jaundice patients.* World J Surg, 2004. **28**(5): p. 475-81.

60. Bennink, R.J., W. de Graaf, and T.M. van Gulik, *Toward a total and regional scintigraphic liver function test enabling assessment of future remnant liver function.* Nucl Med Commun, 2011. **32**(8): p. 669-71.

61. Heger, M., et al., *A clinical perspective on the criteria for liver resection and the use of liver function tests.* World J Surg, 2010. **34**(4): p. 868-9; author reply 870.

62. Hoekstra, L.T., et al., *Physiological and biochemical basis of clinical liver function tests: a review.* Ann Surg, 2013. **257**(1): p. 27-36.

63. Morris-Stiff, G., D. Gomez, and R. Prasad, *Quantitative assessment of hepatic function and its relevance to the liver surgeon.* J Gastrointest Surg, 2009. **13**(2): p. 374-85.

64. Nilsson, H., et al., *Assessment of liver function in primary biliary cirrhosis using Gd-EOB-DTPA-enhanced liver MRI.* HPB (Oxford), 2010. **12**(8): p. 567-76.

65. Schneider, P.D., *Preoperative assessment of liver function.* Surgical Clinics of North America, 2004. **84**(2): p. 355-373.

66. Tralhao, J.G., et al., *Study of perioperative liver function by dynamic monitoring of ICG-clearance.* Hepatogastroenterology, 2012. **59**(116): p. 1179-83.

67. Fan, S.T., et al., *Hospital mortality of major hepatectomy for hepatocellular carcinoma associated with cirrhosis.* Arch Surg, 1995. **130**(2): p. 198-203.

68. Du, Z.G., et al., *An accurate predictor of liver failure and death after hepatectomy: a single institution's experience with 478 consecutive cases.* World J Gastroenterol, 2014. **20**(1): p. 274-81.

69. de Graaf, W., et al., *Increase in future remnant liver function after preoperative portal vein embolization.* Br J Surg, 2011. **98**(6): p. 825-34.

70. de Graaf, W., et al., *(99m)Tc-mebrofenin hepatobiliary scintigraphy with SPECT for the assessment of hepatic function and liver functional volume before partial hepatectomy.* J Nucl Med, 2010. **51**(2): p. 229-36.

71. Dinant, S., et al., *Risk assessment of posthepatectomy liver failure using hepatobiliary scintigraphy and CT volumetry.* J Nucl Med, 2007. **48**(5): p. 685-92.

72. de Graaf, W., et al., *Assessment of future remnant liver function using hepatobiliary scintigraphy in patients undergoing major liver resection.* J Gastrointest Surg, 2010. **14**(2): p. 369-78.

73. Abulkhir, A., et al., *Preoperative portal vein embolization for major liver resection: a meta-analysis.* Ann Surg, 2008. **247**(1): p. 49-57.

74. de Baere, T., A. Denys, and D.C. Madoff, *Preoperative portal vein embolization: indications and technical considerations.* Tech Vasc Interv Radiol, 2007. **10**(1): p. 67-78.

75. Hemming, A.W., et al., *Preoperative portal vein embolization for extended hepatectomy.* Ann Surg, 2003. **237**(5): p. 686-91; discussion 691-3.

76. Imamura, H., et al., *Preoperative portal vein embolization: an audit of 84 patients.* Hepatology, 1999. **29**(4): p. 1099-105.

77. Kianmanesh, R., et al., *Right portal vein ligation: a new planned two-step all-surgical approach for complete resection of primary gastrointestinal tumors with multiple bilateral liver metastases.* J Am Coll Surg, 2003. **197**(1): p. 164-70.

78. Liu, H. and S. Zhu, *Present status and future perspectives of preoperative portal vein embolization.* Am J Surg, 2009. **197**(5): p. 686-90.

79. Pandanaboyana, S., et al., *A systematic review and meta-analysis of portal vein ligation versus portal vein embolization for elective liver resection.* Surgery, 2015. **157**(4): p. 690-8.

80. Guiu, B., et al., *Portal vein embolization before right hepatectomy: improved results using n-butyl-cyanoacrylate compared to microparticles plus coils.* Cardiovasc Intervent Radiol, 2013. **36**(5): p. 1306-12.

81. Kishi, Y., et al., *Is embolization of segment 4 portal veins before extended right hepatectomy justified?* Surgery, 2008. **144**(5): p. 744-51.

82. van Gulik, T.M., et al., *Controversies in the use of portal vein embolization.* Dig Surg, 2008. **25**(6): p. 436-44.

83. Hoekstra, L.T., et al., *Tumor progression after preoperative portal vein embolization.* Ann Surg, 2012. **256**(5): p. 812-7; discussion 817-8.

84. Vigano, L., et al., *Progression while receiving preoperative chemotherapy should not be an absolute contraindication to liver resection for colorectal metastases.* Ann Surg Oncol, 2012. **19**(9): p. 2786-96.

85. Fischer, C., et al., *Chemotherapy after portal vein embolization to protect against tumor growth during liver hypertrophy before hepatectomy.* JAMA Surg, 2013. **148**(12): p. 1103-8.

86. Pamecha, V., et al., *Effect of portal vein embolisation on the growth rate of colorectal liver metastases.* Br J Cancer, 2009. **100**(4): p. 617-22.

87. Spelt, L., et al., *Tumour growth after portal vein embolization with pre-procedural chemotherapy for colorectal liver metastases.* HPB (Oxford), 2015. **17**(6): p. 529-35.

88. Simoneau, E., et al., *Portal vein embolization and its effect on tumour progression for colorectal cancer liver metastases.* Br J Surg, 2015. **102**(10): p. 1240-9.

89. de Baere, T., et al., *Predictive factors for hypertrophy of the future remnant liver after selective portal vein embolization.* Ann Surg Oncol, 2010. **17**(8): p. 2081-9.

90. Yokoyama, Y., M. Nagino, and Y. Nimura, *Mechanisms of hepatic regeneration following portal vein embolization and partial hepatectomy: a review.* World J Surg, 2007. **31**(2): p. 367-74.

91. Lam, V.W., et al., *A systematic review of two-stage hepatectomy in patients with initially unresectable colorectal liver metastases.* HPB (Oxford), 2013. **15**(7): p. 483-91.

92. Hwang, S., et al., *Preoperative Sequential Portal and Hepatic Vein Embolization in Patients with Hepatobiliary Malignancy.* World J Surg, 2015.

93. Jaeck, D., et al., *One or two-stage hepatectomy combined with portal vein embolization for initially nonresectable colorectal liver metastases.* The American Journal of Surgery, 2003. **185**(3): p. 221-229.

94. Adam, R., et al., *Two-stage hepatectomy: A planned strategy to treat irresectable liver tumors.* Ann Surg, 2000. **232**(6): p. 777-85.

95. Adam, R., et al., *Two-stage hepatectomy approach for initially unresectable colorectal hepatic metastases.* Surg Oncol Clin N Am, 2007. **16**(3): p. 525-36, viii.

96. Aloia, T.A. and J.N. Vauthey, *Associating liver partition and portal vein ligation for staged hepatectomy (ALPPS): what is gained and what is lost?* Ann Surg, 2012. **256**(3): p. e9; author reply e16-9.

97. Schadde, E., et al., *ALPPS Offers a Better Chance of Complete Resection in Patients with Primarily Unresectable Liver Tumors Compared with Conventional-Staged Hepatectomies: Results of a Multicenter Analysis.* World J Surg, 2014. **38**(6): p. 1510-9.

98. Masi, G., et al., *Randomized trial of two induction chemotherapy regimens in metastatic colorectal cancer: an updated analysis.* J Natl Cancer Inst, 2011. **103**(1): p. 21-30.

99. Sanoff, H.K., et al., *Five-year data and prognostic factor analysis of oxaliplatin and irinotecan combinations for advanced colorectal cancer: N9741.* J Clin Oncol, 2008. **26**(35): p. 5721-7.

100. Aloia, T.A., *Insights into ALPPS.* Eur J Surg Oncol, 2015. **41**(5): p. 610-1.

101. Dokmak, S. and J. Belghiti, *Which limits to the "ALPPS" approach?* Ann Surg, 2012. **256**(3): p. e6; author reply e16-7.

102. Figueras, J. and J. Belghiti, *The ALPPS Approach: Should We Sacrifice Basic Therapeutic Rules in the Name of Innovation?* World J Surg, 2014. **38**(6): p. 1520-1.

103. Kokudo, N. and J. Shindoh, *How can we safely climb the ALPPS?* Updates Surg, 2013. **65**(3): p. 175-7.

104. Herman, P., et al., *High Mortality Rates After ALPPS: the Devil Is the Indication.* J Gastrointest Cancer, 2015. **46**(2): p. 190-4.

105. Knoefel, W.T., et al., *In situ liver transection with portal vein ligation for rapid growth of the future liver remnant in two-stage liver resection.* Br J Surg, 2013. **100**(3): p. 388-94.

106. Nadalin, S., et al., *Indications and limits for associating liver partition and portal vein ligation for staged hepatectomy (ALPPS). Lessons Learned from 15 cases at a single centre.* Z Gastroenterol, 2014. **52**(1): p. 35-42.

107. Truant, S., et al., *Associating liver partition and portal vein ligation for staged hepatectomy (ALPPS): impact of the inter-stages course on morbi-mortality and implications for management.* Eur J Surg Oncol, 2015. **41**(5): p. 674-82.

108. Schadde, E., et al., *Early survival and safety of ALPPS: first report of the International ALPPS Registry.* Ann Surg, 2014. **260**(5): p. 829-36; discussion 836-8.

109. Alvarez, F.A., et al., *Associating liver partition and portal vein ligation for staged hepatectomy offers high oncological feasibility with adequate patient safety: a prospective study at a single center.* Ann Surg, 2015. **261**(4): p. 723-32.

110. Donati, M., G.A. Stavrou, and K.J. Oldhafer, *Current position of ALPPS in the surgical landscape of CRLM treatment proposals.* World J Gastroenterol, 2013. **19**(39): p. 6548-54.

111. Hasselgren, K., P. Sandstrom, and B. Bjornsson, *Role of associating liver partition and portal vein ligation for staged hepatectomy in colorectal liver metastases: a review.* World J Gastroenterol, 2015. **21**(15): p. 4491-8.

112. Ratti, F., et al., *Defining indications to ALPPS procedure: technical aspects and open issues.* Updates Surg, 2014. **66**(1): p. 41-9.

113. Ratti, F., et al., *Strategies to Increase the Resectability of Patients with Colorectal Liver Metastases: A Multi-center Case-Match Analysis of ALPPS and Conventional Two-Stage Hepatectomy.* Ann Surg Oncol, 2015. **22**(6): p. 1933-42.

114. Schadde, E., et al., *Systematic Review and Meta-Analysis of Feasibility, Safety, and Efficacy of a Novel Procedure: Associating Liver Partition and Portal Vein Ligation for Staged Hepatectomy.* Ann Surg Oncol, 2014.

115. Shindoh, J., et al., *Analysis of the efficacy of portal vein embolization for patients with extensive liver malignancy and very low future liver remnant volume, including a*

comparison with the associating liver partition with portal vein ligation for staged hepatectomy approach. J Am Coll Surg, 2013. **217**(1): p. 126-33; discussion 133-4.

116. Vauthey, J.N. and Y. Mise, *Commentary on "can we improve the morbidity and mortality associated with the associating liver partition with portal vein ligation for staged hepatectomy (ALPPS) procedure in the management of colorectal liver metastases?".* Surgery, 2015. **157**(2): p. 207-10.

117. Oldhafer, K.J., et al., *ALPPS for Patients with Colorectal Liver Metastases: Effective Liver Hypertrophy, but Early Tumor Recurrence.* World J Surg, 2014. **38**(6): p. 1504-9.

118. Schadde, E., et al., *Monosegment ALPPS hepatectomy: extending resectability by rapid hypertrophy.* Surgery, 2015. **157**(4): p. 676-89.

119. Bertens, K.A., et al., *ALPPS: challenging the concept of unresectability--a systematic review.* Int J Surg, 2015. **13**: p. 280-7.

120. Bjornsson, B., T. Gasslander, and P. Sandstrom, *In situ split of the liver when portal venous embolization fails to induce hypertrophy: a report of two cases.* Case Rep Surg, 2013. **2013**: p. 238675.

121. Tschuor, C., et al., *Salvage parenchymal liver transection for patients with insufficient volume increase after portal vein occlusion -- an extension of the ALPPS approach.* Eur J Surg Oncol, 2013. **39**(11): p. 1230-5.

122. Vyas, S.J., et al., *Failure of portal venous embolization. ALPPS as salvage enabling successful resection of bilobar liver metastases.* J Gastrointest Cancer, 2014. **45 Suppl 1**: p. 233-6.

123. de Santibanes, E., F.A. Alvarez, and V. Ardiles, *How to avoid postoperative liver failure: a novel method.* World J Surg, 2012. **36**(1): p. 125-8.

124. Eisenhauer, E.A., et al., *New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1).* Eur J Cancer, 2009. **45**(2): p. 228-47.

125. Clavien, P.A., et al., *The Clavien-Dindo classification of surgical complications: five-year experience.* Ann Surg, 2009. **250**(2): p. 187-96.

126. Dindo, D., N. Demartines, and P.-A. Clavien, *Classification of Surgical Complications.* Annals of Surgery, 2004. **240**(2): p. 205-213.

127. Ekman, M., et al., *Liver uptake function measured by IODIDA clearance rate in liver transplant patients and healthy volunteers.* Nucl Med Commun, 1996. **17**(3): p. 235-42.

128. Simoneau, E., et al., *Portal vein embolization stimulates tumour growth in patients with colorectal cancer liver metastases.* HPB (Oxford), 2012. **14**(7): p. 461-8.

129. Pommier, R., et al., *Colorectal liver metastases growth in the embolized and non-embolized liver after portal vein embolization: influence of initial response to induction chemotherapy.* Ann Surg Oncol, 2014. **21**(9): p. 3077-83.

130. Sturesson, C., I. Keussen, and K.G. Tranberg, *Prolonged chemotherapy impairs liver regeneration after portal vein occlusion - an audit of 26 patients.* Eur J Surg Oncol, 2010. **36**(4): p. 358-64.

131. Covey, A.M., et al., *Combined portal vein embolization and neoadjuvant chemotherapy as a treatment strategy for resectable hepatic colorectal metastases.* Ann Surg, 2008. **247**(3): p. 451-5.

132. Ribero, D., et al., *Portal vein embolization before major hepatectomy and its effects on regeneration, resectability and outcome.* Br J Surg, 2007. **94**(11): p. 1386-94.

133. Sturesson, C., et al., *Importance of thrombocytes for the hypertrophy response after portal vein embolization.* Hepatogastroenterology, 2015. **62**(137): p. 98-101.

134. Cieslak, K.P., et al., *Assessment of Liver Function Using (99m)Tc-Mebrofenin Hepatobiliary Scintigraphy in ALPPS (Associating Liver Partition and Portal Vein Ligation for Staged Hepatectomy).* Case Rep Gastroenterol, 2015. **9**(3): p. 353-60.

135. Truant, S., et al., *Drop of Total Liver Function in the Interstages of the New Associating Liver Partition and Portal Vein Ligation for Staged Hepatectomy Technique: Analysis of the "Auxiliary Liver" by HIDA Scintigraphy.* Ann Surg, 2016. **263**(3): p. e33-4.

136. Lau, L., C. Christophi, and V. Muralidharan, *Intraoperative functional liver remnant assessment with indocyanine green clearance: another toehold for climbing the "ALPPS".* Ann Surg, 2015. **261**(2): p. e43-5.

137. Cressman, D.E., et al., *Liver failure and defective hepatocyte regeneration in interleukin-6-deficient mice.* Science, 1996. **274**(5291): p. 1379-83.

138. Ohira, H., et al., *Interleukin-6 induces proliferation of rat hepatocytes in vivo.* J Hepatol, 1996. **25**(6): p. 941-7.

139. Hernandez-Alejandro, R., et al., *Can we improve the morbidity and mortality associated with the associating liver partition with portal vein ligation for staged hepatectomy (ALPPS) procedure in the management of colorectal liver metastases?* Surgery, 2015. **157**(2): p. 194-201.

140. Schadde, E., et al., *Hypoxia of the growing liver accelerates regeneration.* Surgery, 2016.

141. Croome, K.P., et al., *Is the liver kinetic growth rate in ALPPS unprecedented when compared with PVE and living donor liver transplant? A multicentre analysis.* HPB (Oxford), 2015. **17**(6): p. 477-84.

142. Adam, R., et al., *Outcome after associating liver partition and portal vein ligation for staged hepatectomy and conventional two-stage hepatectomy for colorectal liver metastases.* Br J Surg, 2016. **103**(11): p. 1521-9.

143. Olthof, P.B., et al., *Survival after associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) for advanced colorectal liver metastases: A case-matched comparison with palliative systemic therapy.* Surgery, 2016.

144. Geisel, D., et al., *Gd-EOB-DTPA-enhanced MRI for monitoring future liver remnant function after portal vein embolization and extended hemihepatectomy: A prospective trial.* Eur Radiol, 2016.