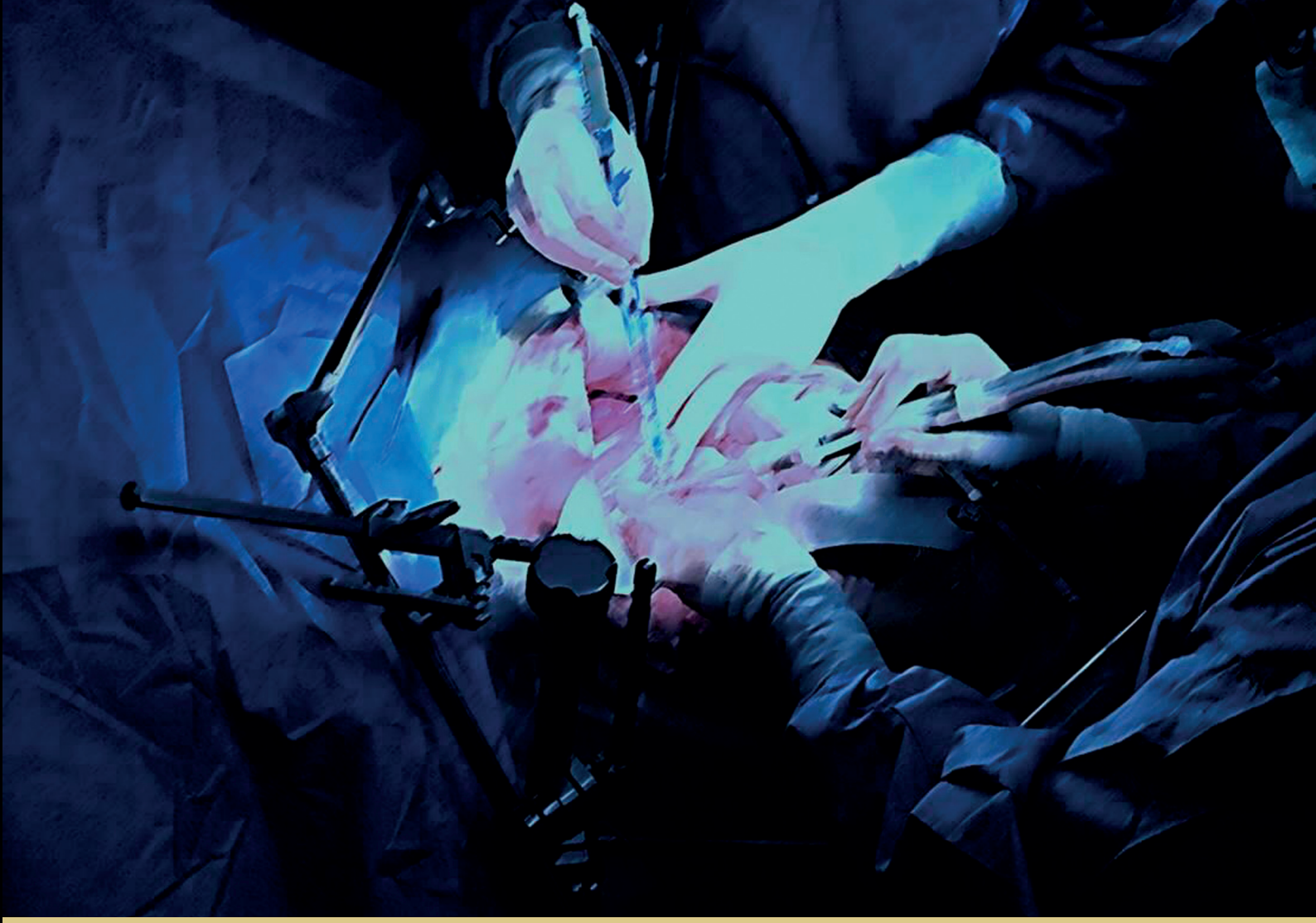

Henrique Miguel Marques Bom Borges Alexandrino

ENERGY FOR LIVER REGENERATION: MITOCHONDRIAL BIOENERGETICS AND THE PATHOGENESIS OF POSTHEPATECTOMY LIVER DYSFUNCTION

Doctoral Thesis in Health Sciences, branch of Medicine, supervised by Prof. Doutor Francisco José Franquera Castro e Sousa, MD, PhD and by Prof. Doutor Carlos Manuel Marques Palmeira, PhD and presented to the Faculty of Medicine of the University of Coimbra

Março, 2017

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Universidade de Coimbra

Henrique Miguel Marques Bom Borges Alexandrino

Energy for Liver Regeneration: Mitochondrial Bioenergetics and the Pathogenesis of Posthepatectomy Liver Dysfunction

Energia para a Regeneração Hepática: Bioenergética Mitocondrial e a Patogénese da Insuficiência Hepática Pós-Hepatectomia

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À memória dos meus Avós

Rosa e Urbano

“We make our world significant by the courage of our questions and the depth of our answers”

Carl Sagan, in “Cosmos” 1980

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Abstract / Resumo

Abstract

Clinical success of hepatectomy relies on the liver's unique capacity to regenerate, a highly energy-dependent process. When this capacity is surpassed Posthepatectomy Liver Failure (PHLF) ensues, resulting in increased morbidity and mortality.

Mitochondria are the powerhouses of the eukaryote cell and decision-makers of cell death. Mitochondrial metabolism supplies the energy for liver regeneration, but the clinical consequences of mitochondrial dysfunction in posthepatectomy morbidity and liver function are presently unknown. Other unresolved issues are the effects of chemotherapy-associated liver injury (CALI) on outcome, as well as the possible contribution of bioenergetic dysfunction to the pathogenesis of CALI. Finally, two-stage hepatectomies rely on an extremely rapid and significant inter-stages regenerative response, but the energetic adaptations taking place in the future liver remnant (FLR) are largely unknown. Mitochondrial oxidative phosphorylation and respiration are key events in cellular energy metabolism and can be directly measured in liver biopsies.

In this Doctoral Thesis the following objectives were pursued: 1) Review the role of energy metabolism in liver regeneration; 2) Determine whether changes in mitochondrial function correlate with clinical outcome in Humans undergoing hepatectomy; 3) Assess the impact of hepatic pedicle clamping (HPC) on intraoperative mitochondrial function; 4) Evaluate the impact of chemotherapy-induced hepatotoxicity on postoperative morbidity and the putative role of mitochondrial dysfunction in its pathogenesis; 5) Investigate the bioenergetics adaptations underlying the enhanced regenerative response taking place in two-stage hepatectomies.

For the first objective, a non-systematic review of relevant published material in the English language was conducted. Reference lists were cross-checked for further relevant publications. Mitochondrial oxidative phosphorylation was summarily reviewed, as well as the role of mitochondria in both apoptotic and necrotic cell death. The key role of mitochondrial metabolism in the cellular events leading to liver regeneration was confirmed.

For the second and third objectives, both experimental and clinical works were performed. First, a prospective study of patients undergoing hepatectomy for diverse indications (N=30) was conducted. Liver biopsies were performed in two distinct moments, at the beginning and at the end of surgery. Mitochondria were isolated and membrane potential and respiration were measured. Mitochondrial lag phase, reflecting the time required for adenosine diphosphate phosphorylation, presented high sensitivity and specificity for prediction of PHLF; a finding previously unreported in the scientific literature. Several key markers of postoperative liver function presented significant correlations with intraoperative fluctuations in mitochondrial membrane potential and respiration. An experimental study (N=35 male Wistar rats) was also outlined to explore the effect of 70% hepatectomy with HPC on energy metabolism, liver function and injury. In both studies, clinical and experimental, HPC was associated with depressed mitochondrial function.

The fourth objective was addressed with two different methods. First, a clinical and pathologic review of 140 patients undergoing hepatectomy for colorectal cancer liver metastases was performed to look into the incidence, pathological spectrum and clinical consequences of CALI. Sinusoidal obstruction syndrome (SOS) was present in 52% of patients and independently associated with overall and liver-specific morbidity. Secondly, an experimental study (N=12 male Wistar rats), attempted to replicate a previously described animal model of SOS. Hepatectomy with HPC was performed to explore the possible link of mitochondrial dysfunction in the pathogenesis of posthepatectomy liver dysfunction in CALI. Although the characteristic histologic findings of SOS were not found, chemotherapy-treated animals presented evidence of disturbed hepatocellular bioenergetics, namely longer lag phase and increased mitochondrial size.

Finally, another original prospective clinical study was conducted on patients undergoing the Associating Liver Partition and Portal Vein Ligation for Staged Hepatectomy (ALPPS) procedure (N=5). In this study, mitochondrial membrane potential and respiration were measured as well as gene expression profile. An inter-stages increase in energetic capacity was demonstrated in the FLR, as well as a strong and significant correlation of energy status with inter-stages volume growth. An increased expression of several genes associated with liver regeneration (*Augmenter of*

Liver Regeneration; Small heterodimer partner; Signal Transducer and Activator of Transcription 3), mitochondrial biogenesis (*Peroxisome proliferator-activated receptor gamma coactivator 1-alpha*) and energy metabolism (*Cytochrome oxidase subunit I; Nicotinamide phosphoribosyltransferase*) was also described. To our knowledge, this is the first ever documentation of adaptations in energy metabolism in two-stage hepatectomies in Humans.

In conclusion, not only is Liver Regeneration highly dependent on energy metabolism, but also mitochondrial dysfunction was demonstrated to be involved in the pathogenesis of PHLF. The clinical relevance of mitochondrial bioenergetics in clinical liver resection deserves further exploration, with particular emphasis on the accurate perioperative staging of liver energy status as well as energy-conditioning therapies aiming at decreasing morbidity and mortality.

Resumo

O sucesso clínico da Cirurgia Hepática de exérese depende da capacidade singular do fígado para regenerar, um processo altamente dependente de energia. Quando esta capacidade é ultrapassada, desencadeia-se a Insuficiência Hepática Pós-Hepatectomia (PHLF), com consequente morbi-mortalidade.

As mitocôndrias são as responsáveis pela produção de energia nas células eucariotas e decisores-chave na morte celular. O metabolismo mitocondrial fornece energia para a regeneração hepática, mas desconhecem-se as consequências clínicas da disfunção mitocondrial sobre a morbilidade e função hepatocelular pós-hepatectomia. Outra questão que ainda carece definição é o efeito da lesão hepática associada à quimioterapia (CALI) nos resultados clínicos, bem como o possível papel do metabolismo energético na sua patogénese. Finalmente, as hepatectomias iterativas dependem de uma importante resposta regenerativa, mas as adaptações energéticas que ocorrem no fígado remanescente (FLR) são desconhecidas. A fosforilação oxidativa e a respiração mitocondriais são eventos-chave no metabolismo energético e podem ser medidas directamente em biópsias hepáticas.

Nesta dissertação doutoral foram perseguidos os seguintes objectivos: 1) Revisão do papel do metabolismo energético na regeneração hepática; 2) Determinar a relação entre a função mitocondrial e os resultados clínicos após hepatectomia no Humano; 3) Avaliar o impacto da clampagem do pedículo hepático (HPC) sobre a função mitocondrial; 4) Aferir as consequências da hepatotoxicidade induzida pela quimioterapia na morbilidade pós-hepatectomia, bem como o papel da disfunção mitocondrial na sua patogénese; 5) Investigar as adaptações bioenergéticas subjacentes à marcada resposta regenerativa que ocorre nas hepatectomias iterativas.

Para o primeiro objectivo foi realizada uma revisão não sistemática da literatura em língua inglesa, com pesquisa de artigos presentes nas listas de referências. A fosforilação oxidativa foi sumariamente revista, bem como o papel das mitocôndrias nos processos de apoptose e necrose. Foi assim confirmado o papel do metabolismo mitocondrial nos eventos celulares que culminam na regeneração hepática.

Para responder ao segundo e terceiro objectivos foram realizados um estudo experimental e outro clínico. Em primeiro lugar, foram estudados prospectivamente doentes submetidos a hepatectomia por indicações diversas (N=30), com realização de biópsias hepáticas intra-operatórias, no início e no final da intervenção, e medição do potencial de membrana e respiração mitocondriais. A "*lag phase*" mitocondrial, o tempo necessário à completa fosforilação da adenosina difosfato, apresentou uma elevada sensibilidade e especificidade na previsão de PHLF, um achado nunca antes reportado. Vários marcadores de função hepatocelular pós-operatória apresentaram correlações significativas com a flutuação peri-operatória do potencial de membrana e respiração mitocondriais. Para além disso, um estudo experimental (N=35 ratos Wistar machos) foi desenhado para explorar o efeito da hepatectomia de 70% com HPC no metabolismo energético e na função e lesão hepática; em ambos os estudos foi confirmado o efeito deletério da HPC sobre a função mitocondrial.

A resposta ao quarto objectivo decorreu de dois modos distintos. Em primeiro lugar, foi realizada uma revisão clínica e patológica de 140 doentes submetidos a hepatectomia por metástases de cancro colo-rectal, com o intuito de definir a incidência, espectro patológico e consequências clínicas da CALI. O síndrome de obstrução sinusoidal (SOS), presente em 52% dos doentes, associou-se de forma independente com a morbilidade global e relacionada com o fígado. Em segundo lugar, procurou-se replicar um modelo animal de SOS num estudo experimental (N=12 ratos Wistar machos). Neste, realizaram-se hepatectomias com HPC de modo a explorar uma possível conexão entre disfunção mitocondrial e disfunção hepatocelular na CALI. Embora não tenham sido obtidas as lesões histológicas típicas do SOS, os animais tratados com quimioterapia apresentaram significativa perturbação da bioenergética hepatocelular, nomeadamente "*lag phase*" mais prolongada e aumento do tamanho mitocondrial.

Finalmente, outro estudo clínico prospectivo original foi conduzido em doentes submetidos a Associação de Laqueação Portal com Secção Parenquimatosa para Hepatectomia a dois tempos (ALPPS) (N=5); neste estudo foi realizada a avaliação da bioenergética mitocondrial e a análise da expressão génica. Verificou-se um aumento da função mitocondrial entre estadios, bem como uma correlação forte e significativa entre o status energético e o crescimento volumétrico do FLR. Foi ainda objectivado um

aumento da expressão de diversos genes associados à regeneração hepática (*Augmenter of Liver Regeneration; Small heterodimer partner*; *Signal Transducer and Activator of Transcription 3*), biogénese mitocondrial (*Peroxisome proliferator-activated receptor gamma coactivator 1-alpha*) e metabolismo energético (*Cytochrome oxidase subunit I; Nicotinamide phosphoribosyltransferase*). Tanto quanto nos é dado saber, trata-se da primeira vez que, no Homem, se relatam adaptações do metabolismo energético em hepatectomias iterativas.

Em conclusão, poder-se-á dizer que não só a regeneração hepática é um processo altamente dependente de energia, mas, também, que a função mitocondrial assume um papel relevante na fisiopatologia da PHLF. A possível relevância clínica da bioenergética mitocondrial na Cirurgia Hepática merece exploração mais detalhada, com particular ênfase na correcta aferição da capacidade energética peri-operatória, mas também na possibilidade de melhorar os resultados, com o uso de terapêuticas de condicionamento energético do FLR, reduzindo a morbi-mortalidade.

List of abbreviations

5-FU	5-fluorouracil
AALF	Acetaminophen-induced acute liver failure
ADP	Adenosine diphosphate
AKBR	Arterial Ketone Body Ratio
ALPPS	Associating Liver Partition and Portal Vein Ligation for Staged Hepatectomy
ALR	Augmenter of Liver Regeneration
ALT	Alanine aminotransferase
ANT	Adenine Nucleotide Translocator
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
ATP/Pi	Adenosine triphosphate to phosphate ratio
BDL	Bile duct ligation
BLAST	Basic Local Alignment Search Tool
BSA	Bovine serum albumin
CACT	Carnitine-acetylcarnitine translocase
CALI	Chemotherapy-associated liver injury
CASH	Chemotherapy-associated steatohepatitis
cDNA	Complementary deoxyribonucleic acid
CECT	Contrast-enhanced computed tomography
CK	Creatine kinase
COX	Cytochrome oxidase
COX1	Cytochrome oxidase subunit I
COX4	Cytochrome oxidase subunit IV
CRLM	Colorectal cancer liver metastases
CT	Computed tomography
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid

EGF	Epidermal growth factor
EGTA	Ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid
ETC	Electron transport chain
FA	Fatty acids
$FADH_2$ or FAD	Flavin adenine dinucleotide
FLR	Future liver remnant
FOLFIRI	Association of 5-fluoruracil with irinotecan
FOLFOX	Association of 5-fluoruracil with oxaliplatin
FXR	Farnesoid X receptor
GLDH	Glutamate dehydrogenase
GLP-1	Glucagon-like peptide 1
GR	Growth rate
GSH	Glutathione
H_2DCFDA	2′,7′-dichlorodihydrofluorescein diacetate
HABR	Hepatic Artery Buffer Response
H&E	Haematoxylin and Eosin
HBO	Hyperbaric oxygen
HCC	Hepatocellular carcinoma
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HGF	Hepatocyte Growth Factor
HPC	Hepatic Pedicle Clamping
HR	Hazard ratio
ICG	Indocyanine Green
IL-6	Interleukin 6
INR	International Normalized Ratio
IRI	Ischemia-reperfusion injury
IQR	Interquartile range
ISGLS	International Study Group for Liver Surgery

KGR	Kinetic growth rate
LDH	Lactate dehydrogenase
LOS	Length of stay
MaHp	Major hepatectomy
MARS	Molecular Adsorbent Recirculating System
MCT	Monocrotaline
MELD	Model for End Stage Liver Disease
miHp	Minor hepatectomy
MPT	Mitochondrial permeability transition
mtDNA	Mitochondrial deoxyribonucleic acid
NADH or NAD+	Nicotinamide adenine dinucleotide
NAMPT	Nicotinamide phosphoribosyltransferase
NCBI	National Center for Biotechnology Information
NCT	Neoadjuvant chemotherapy
NIM811	N-methyl-4-isoleucine cyclosporine
NRF-1	Nuclear respiratory factor 1
NRF-2	Nuclear factor erythroid 2-related factor 2
OR	Odds ratio
PCR	Polymerase chain reaction
PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PHLF	Posthepatectomy Liver Failure
PVA	Portal vein arterialization
PVE	Portal vein embolization
PVL	Portal vein ligation
PVP	Portal vein pressure
RCR	Respiratory Control Ratio
RNA	Ribonucleic acid
ROS	Reactive oxygen species

RR	Relative risk
SAMe	S-adenosyl-L-methionine
SD	Standard deviation
SEC	Sinusoidal endothelial cell
SEM	Standard error of the mean
sFLR	Standardized future liver remnant
SFSS	Small-for-size syndrome
SH	Steatohepatitis
SHP	Small heterodimer partner
SirT1	Sirtuin 1
SOS	Sinusoidal obstruction syndrome
STAT3	Signal Transducer and Activator of Transcription 3
TEM	Transmission electron microscopy
TFAM	Mitochondrial transcription factor A
TGF-α	Transforming growth factor alpha
TNF-α	Tumor Necrosis Factor α
TPP	Tetraphenylphosphonium
TRAIL-R1	Tumor necrosis factor-related apoptosis-inducing ligand receptor 1
TRAIL-R2	Tumor necrosis factor-related apoptosis-inducing ligand receptor 2
UCP-2	Uncoupling protein 2

Foreword

The current Doctoral Thesis revolves around the subject of Energy, which is defined as "*the capacity of a system to perform work*" (Koolman, Roehm, 2005). The Liver is the master regulator of complex multicellular animals and the inexhaustible guardian of metabolic equilibrium. Either storing energy in the fed state, or releasing it for other organs in the fast state, the Liver also synthetizes most of plasma proteins, metabolizes and excretes endogenous and exogenous organic molecules in the bile, while maintaining a discrete, yet vital, immune function. Furthermore, the Liver has the extraordinary capacity to recuperate after insults, restoring its original size and function. Such is the Liver's work.

This potential for regeneration is the cornerstone of modern Hepatic Surgery, whereby patients with primary and secondary malignant neoplasms are amenable to cure after resection of a significant fraction of liver parenchyma, without resulting in any permanent curtailment of function. Notwithstanding, Liver Regeneration is on occasion inadvertently pushed beyond its limits. This results in one of the most ominous complications of liver resection, Posthepatectomy Liver Failure, culminating in the collapse of the metabolic building of the entire organism.

But what fuels Liver Regeneration? Where does the Liver obtain the energy to replenish its cell population while ensuring the metabolic stability of the internal milieu? More importantly, what energetic adaptations occur after liver resection and how is the energy status related to the postoperative outcome? And how does hepatic pedicle clamping influence liver energy status? What is the effect of chronic liver disease on energy metabolism and on the response to surgical insult? Moreover, what energy-based measures could be used for earlier detection, and even prevention, of the dreaded Posthepatectomy Liver Failure? And finally, what is the energy source for the short-interval two-stage hepatectomies, one of the most dramatic and risky challenges that modern Hepatic Surgery places to Liver Regeneration? These were the research questions underlying this project.

Soon a singular intracellular organelle assumed a pivotal role. Like the Liver, Mitochondria are tireless providers of energy. By breaking down the carbon skeleton of organic molecules in the presence of oxygen, Mitochondria supply heterotrophic eukaryote cells with the fuel for all of Life's functions. Additionally, they are key regulators of cell death. If they malfunction the cell succumbs, relinquishing all activity.

So, as the Liver is the metabolic coordinator of the Body, so are Mitochondria of the Cell.

Under these postulates, research was conducted in a translational perspective. Questions arose in the clinical setting, where rare, yet vexing cases of posthepatectomy liver dysfunction ignited an intellectual unrest. The interrogations led to the laboratory, where more profound insights were investigated, giving rise to novel problems and innovative perspectives. And the quest for answers began. Theories were formulated and tested. Results were compared and discussed. New information was collected and summarized. And the end result of this work is: "*Energy for Liver Regeneration: Mitochondrial Bioenergetics and the Pathogenesis of Posthepatectomy Liver Dysfunction*".

This Thesis is thus divided into four chapters. In the introductory chapter, Chapter I - "*Mitochondria and Liver Regeneration: Review of the Role of Energy Metabolism in the Pathophysiology of Posthepatectomy Liver Failure*", the evidence for the contribution of mitochondria in liver regeneration is scrutinized. Relevant scientific literature, both in the fields of Basic and Applied sciences, was searched and critically revised, with emphasis on the ramifications of mitochondrial function in the biological processes occurring after liver resection. The clinical pertinence of the subject was highlighted, mostly on three distinct tracks. First, as a growing proportion of patients undergo resection of a chronically diseased liver parenchyma, the relevance of energy status in the setting of diverse chronic hepatic disorders was explored. Secondly, diagnostic markers of bioenergetics derangement for the earlier and more sensitive detection of postoperative liver dysfunction were examined. And finally, the exhilarant possibility of novel mitochondrial-based therapies for the improvement of posthepatectomy outcome was reviewed.

In Chapter II - "*Mitochondrial Bioenergetics, Hepatic Pedicle Clamping and Posthepatectomy Liver Dysfunction*", original scientific data are displayed, in the search for a nexus between energy status and clinical results of hepatectomy. Both clinical and experimental works are detailed, and a definite link for mitochondrial homeostasis in the liver's recovery after ischemic and surgical insult is explored. And mitochondrial oxidative phosphorylation is for the first time authenticated as a relevant vector in the patient's clinical outcome.

Given the high incidence of colorectal cancer in developed nations, the prevalence of liver metastases, as well as the definite benefits for their resection on patient outcome, this Thesis was compelled to interrogate on this subject. In particular, on a novel form of liver impairment, Chemotherapy-Associated Liver Injury. This was the leitmotif for Chapter III - "*Chemotherapy-Associated Liver Injury and Posthepatectomy Morbidity: Possible contribution of Mitochondrial Dysfunction to the Pathogenesis of Sinusoidal Obstruction Syndrome*". As evidence emerges for a significant deterioration of liver reserve in this setting, a putative link with deranged energy homeostasis was explored. An original retrospective clinical study on the prevalence, pathologic spectrum and clinical consequences of chemotherapy-induced parenchymal injury was conducted and presented. And an experimental model was conceptualized to investigate a vinculum with mitochondrial dysfunction, with the results detailed.

Although the indications for hepatectomy are expanding, the limits of resection were at a standstill until recently, when a novel cutting-edge approach in the Liver surgeons' armamentarium emerged. Drawing on the liver's notable capacity to regenerate, and sometimes tipping this delicate equilibrium beyond its limits, the Associating Liver Partition with Portal Vein Ligation for Staged Hepatectomy was inadvertently discovered and developed. This remarkable technique undoubtedly relies on the extremes of liver regenerative capacity, and possibly also on the bare limits of energy homeostasis. This was again the focus of original clinical research unveiled in the final chapter, Chapter IV – "*Bioenergetic Adaptations of the Liver in the ALPPS Procedure: How Liver Regeneration Correlates with Mitochondrial Energy Status*". Here mitochondrial metabolism proved, once again, to be a ponderous factor in clinical outcome.

A dissertation on the most meaningful findings of this Thesis, as well as a discussion on the possible avenues to pursue in further research is laid out in the *Concluding Remarks* section. And illustrating several salient aspects of Bioenergetics in Liver Surgery, a pictorial essay of original photographs is presented in the *Colour Plate* section.

With this Thesis, a Surgeon-Investigator's effort is not complete, however. Much more remains to be explored. And the enthralling encounter of two fascinating

disciplines – Bioenergetics and Liver Surgery – will surely give rise to new questions; and to the pursuit for new answers. Energy will surely be devoted to this work!

Coimbra, March 2017

Chapter I - Mitochondria and Liver Regeneration: Review of the Role of Energy Metabolism in the Pathophysiology of Posthepatectomy Liver Failure

Introduction

Liver resection, founded on anatomical principles [1], is the only curative therapy for most patients with hepatobiliary malignancies [2–6]. Resection of a substantial amount of liver mass is only possible due to the liver's remarkable capacity to regenerate [7]. However, when this process is hampered Posthepatectomy Liver Failure (PHLF) ensues. PHLF is a severe complication of liver resection, with incidences ranging between 2.1 and 10.6% [8–10], resulting not only in perioperative mortality of up to 54% [11], but also severe morbidity, long-term deterioration in liver function and decreased long-term survival [12,13]. PHLF is characterized by jaundice, ascites, encephalopathy and is usually complicated with renal dysfunction and sepsis [9,14].

The mechanisms behind liver regeneration have been the focus of intense research in the fields of Biology and Clinical Medicine [15–18]. Being a highly energy dependent chain of events, liver regeneration is influenced by the energy status of the main parenchymal cell – the hepatocyte. Mitochondria are the powerhouses of eukaryote cells and key players in cell death. As such, they play a major role in the organ's response to major resection. Although this subject has been the focus of investigation in the past, recent findings have led to a renewal of interest on disordered bioenergetics in liver surgery [19–22]. And even more so, as the most upstream mechanisms that trigger liver regeneration are slowly but surely unravelled, a definite role for energy balance is gaining importance, putting the spotlight on mitochondria as one of the master regulators of liver regeneration [23–26].

In this Chapter we will review the energetic mechanisms fuelling liver regeneration, with emphasis on the role of mitochondria in energy homeostasis and cell death. We will scrutinize the experimental and clinical evidence supporting a major role for mitochondria in the liver's response to resection, as well as the relevance of mitochondrial derangement in the pathophysiology of PHLF. Furthermore, as a significant proportion of patients undergoing hepatectomy have chronic liver diseases, namely cirrhosis, biliary obstruction, steatosis or sinusoidal obstruction syndrome, which are at increased risk of PHLF, we will recapitulate the disordered bioenergetics in the pathophysiology of these conditions. We will also look into the possibility of

accurately predicting postoperative liver failure with non-invasive markers of mitochondrial dysfunction, ultimately allowing an earlier diagnosis of PHLF and the timely institution of therapy. Finally, we will elaborate on the evidence for a definitive role of boosting energetic status of the liver parenchyma in improving the clinical results of hepatectomy.

1. Mitochondria – The Powerhouse of Eukaryote cells

Mitochondria are singular organelles. They are descendent of α-proteobacteria perfectly integrated with their host eukaryote cells, recapitulating one of the most ancient symbiotic relationships of life on planet Earth and propelling eukaryotes' evolution into complex multicellular organisms [27]. They provide over 90% of the energy supply under aerobic conditions and are also key regulators of intracellular calcium and programmed cell death [28]. Like their prokaryotic ancestors, mitochondria are composed of an outer and an inner membrane (separated by the intermembrane space) and a matrix containing a complex enzymatic machinery and a circular deoxyribonucleic acid (DNA) molecule.

Since the liver is the hub of whole-body homeostasis, it is no surprise that there is a constant need for energy in liver cells to perform the diverse anabolic and catabolic reactions involving carbohydrate, lipid, protein, purine and xenobiotic metabolism. The energy for this, in the form of adenosine tri-phosphate (ATP), is provided by mitochondria, through the process of oxidative phosphorylation. In aerobic conditions acetyl-coenzyme A (resulting from glycolysis and from β-oxidation of free fatty acids) is completely oxidised to carbon dioxide in the tricarboxylic acid cycle, in which two coenzymes are reduced: nicotinamide adenine dinucleotide (NAD+) and flavin adenine dinucleotide (FAD) [29]. Both β-oxidation of free fatty acids and the tricarboxylic acid cycle take place in the mitochondrial matrix. The reduced forms of NAD+ and FAD, NADH and $FADH_2$ respectively, transfer their electrons sequentially to the enzyme complexes I to IV of the electron transport chain (ETC), in the mitochondrial inner membrane, to the final acceptor, molecular oxygen, yielding water. During electron transport, protons (H^+) are pumped into the intermembrane space, creating an electrochemical gradient across the mitochondrial inner membrane (proton-rich and

positively charged in the intermembrane space, versus proton-poor and electro-negative in the matrix). The movement of protons back to the mitochondrial matrix is coupled to ATP synthesis by complex V, F_1F_0 ATP-synthase. This enzyme uses the energy of proton movement across the gradient for phosphorylation of adenosine di-phosphate (ADP), generating ATP. The net yield of these reactions is the production of 32 mol of ATP for every mol of glucose [30].

Oxidative phosphorylation is an extremely efficient process, because energy transfer occurs in small steps, with several intermediary molecules and without much dissipation of energy as heat. In spite of this, the transfer of electrons is sometimes incomplete, leading to the production of reactive oxygen species. Reactive oxygen species (ROS) is a collective term that broadly describes a variety of molecules and free radicals (chemical species with one unpaired electron) derived from molecular oxygen: superoxide, singlet oxygen, hydrogen peroxide, and hydroxyl radical. Production of ROS is a physiologic event at basal levels and deleterious consequences are usually prevented by several cellular antioxidant defence mechanisms, such as glutathione, superoxide dismutase and catalase [31]. However, excessive production of ROS (for instance in conditions of ischemia-reperfusion injury) can overwhelm these defences and cause cell damage. Mitochondria are not only the producers but also the main victims of increased ROS formation. Two mitochondrial elements are particularly susceptible to oxidative stress: DNA and inner membrane lipids. Mitochondrial DNA (MtDNA) is extremely sensitive to oxidative damage as it is located close to the inner membrane (where most ROS are formed), lacks protective histones and has incomplete repair mechanisms. Since it encodes 13 essential proteins for the ETC, mutations in MtDNA can severely impair electron transport, propagating the vicious circle of energetic dysfunction and oxidative damage. Lipid peroxidation by ROS, in particular cardiolipin, can further compromise ETC function [32].

Besides the obvious contribution of mitochondrial dysfunction in cell death by necrosis (ATP depletion causes failure of ATP-dependent ionic pumps and osmotic cell swelling and death), mitochondria are also involved in apoptosis. In conditions of mitochondrial calcium overload, especially during ischemia-reperfusion injury (IRI), a non-selective conductive pore (the mitochondrial permeability transition – MPT) is formed in the mitochondrial outer membrane, allowing the leakage of protons (with

subsequent reduced energy efficiency) and cytochrome c into the cytoplasm thus activating the caspase-3 cell death pathway [33].

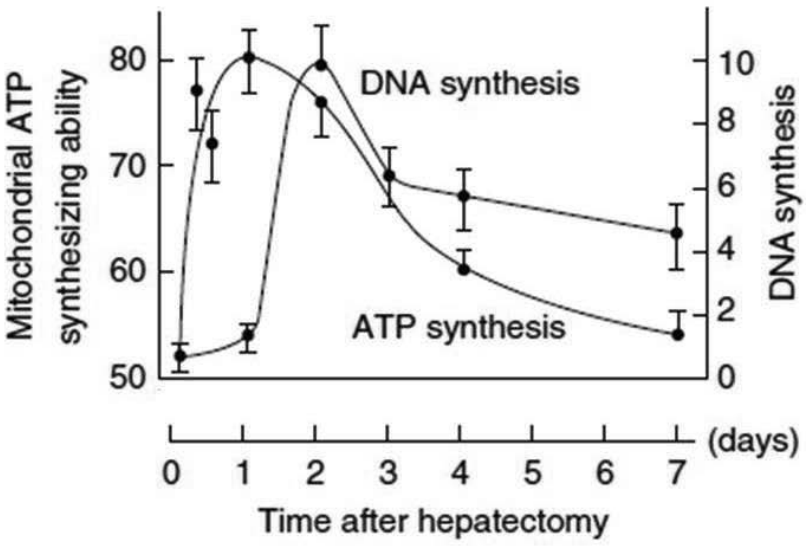
Mitochondrial quality is tightly regulated by a delicate balance between formation of new mitochondria, in the process of mitochondrial biogenesis; and degradation by autophagy. Several genes and transcription factors are responsible for the tight regulation of mitochondrial biogenesis, namely the mitochondrial transcription factor A (TFAM) and Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), ensuring a coordinated collaboration between the mitochondrial 13-gene DNA and the nuclear genome [34,35]. Mitochondrial autophagy or mitophagy, is the process by which old or damaged mitochondria are engulfed in autophagosomes and trafficked to lysosomes, thus maintaining a healthy and functioning mitochondrial pool [36,37]. Remarkably, liver mitochondria have higher turnover rates than mitochondria in other tissues [38].

2. Liver Regeneration – A highly energetic cellular process

Liver regeneration is a highly energy dependent process (Figure 1.1). After hepatectomy the regenerative stimulus causes hepatocytes to undergo cell cycle progression, DNA replication and protein synthesis, processes that require a large amount of energy [15], mostly derived from β-oxidation of fatty acids [39]. Although the increase in ATP synthesis precedes the peak in DNA synthesis [40,41], the net result is a significant drop in liver ATP, more pronounced the more extended the resection of parenchyma, as more energy is required for the diverse anabolic reactions of cell growth and division. In fact, as early as 30 seconds after hepatectomy there is a 50% decrease in liver ATP stores in rodents [24]. The decrease in ATP reaches a nadir at 48 hours but is expected to fully recover by the fifth day. Likewise, there is a decrease in ATP to phosphate (ATP/Pi) ratio, that significantly correlates with postoperative liver function and markers of cellular proliferation [42]. Although the evidence for this is mostly experimental, Mann et al, using ^{31}Phosphorus Magnetic Resonance Spectroscopy, demonstrated that hepatectomy in human subjects was also associated with an early fall in ATP/Pi ratio [43].

Figure 1.1 - Bioenergetics and liver regeneration

Serial changes in ATP and DNA synthesis after hepatectomy. DNA synthesis, a key event in cell cycle progression, is preceded by important metabolic adaptations of liver cells, providing the energy for liver regeneration. As most of the energy produced in liver cells is derived from the aerobic β-oxidation of free fatty acids by mitochondria, liver regeneration is thus dependent on a constant oxygen supply as well as on adequate mitochondrial homeostasis (reproduced from Ozawa et al [41], with permission).



In order to sustain the increased requirements of ATP to fuel liver regeneration, hepatocytes undergo a series of metabolic adaptations. In the first hours after hepatectomy there is an increase in mitochondrial DNA and RNA in the remnant liver [44], augmented expression of several enzymes involved in electron transport and β-oxidation of fatty acids (FA's), such as cytochrome c oxidase and carnitine *O*-palmitoyltransferase [45]. Ultimately this leads to an overall overexpression of the energy-producing enzymatic machinery in the first two to four days after hepatectomy [46]. In a rodent model of 50% hepatectomy, Cao et al demonstrated that of the 87 different proteins expressed during liver regeneration, 25 were mitochondrial proteins, especially involved in carbohydrate and lipid metabolism [47]. Interestingly, after resection hepatocytes also overexpress the thyroid hormone receptor β-2 [45], making them more sensitive to the action of thyroid hormone, triiodothyronine, a key inducer of the β-F1 subunit of ATP synthase and of mitochondrial biogenesis [48,49]. The importance of mitochondrial biogenesis in liver regeneration is further demonstrated by the role played by mitochondrial topoisomerase 1, a key enzyme for the replication of the circular 13-gene mitochondrial DNA. In a model of toxic-induced liver injury,

knock-out mice for this enzyme presented with decreased mitochondrial DNA, lower activities of ETC complexes I and IV and impaired hepatocyte replication [50].

These adaptations are paramount for the increased metabolic demand of liver regeneration, as the recovery in energy status is preceded by an enhanced liver oxygen consumption, which in turn is followed by the peak in DNA replication [51]. As a proof of concept of the importance of hepatic energy status in liver regeneration, Satoh et al, in an elegant experiment, used knock-in mice expressing creatine kinase (CK) in liver cells. CK is normally expressed in skeletal and cardiac muscle, and in brain tissue, but not in the liver. It constitutes an alternate source for adenosine triphosphate (ATP) production by the transfer of a high-energy phosphate from creatine phosphate to ADP. In this experiment, mice with liver expression of CK were fed either a high-creatine diet or a control diet. After 70% hepatectomy, creatine-fed animals had higher hepatic ATP synthesis and displayed increased bromodeoxyuridine incorporation and liver weight gain, versus CK-positive controls with normal diet [52].

Since the availability of ATP is ultimately dependent upon the proper function of mitochondria, it is expected that enhanced mitochondrial function would improve liver regeneration. In fact, this is well illustrated by two experimental works that used N-methyl-4-isoleucine cyclosporine (NIM811), a known inhibitor of mitochondrial membrane permeability transition (MPT). Both in the setting of extended hepatectomy [20] and small-for-size liver transplant [53], NIM811 inhibited the MPT, preserved liver energy status and hepatocellular function and improved survival.

Having summarized the biological link of energy availability to cell proliferation in liver regeneration, we will now discuss the evidence for a definite role of bioenergetics dysfunction in the pathophysiology of PHLF.

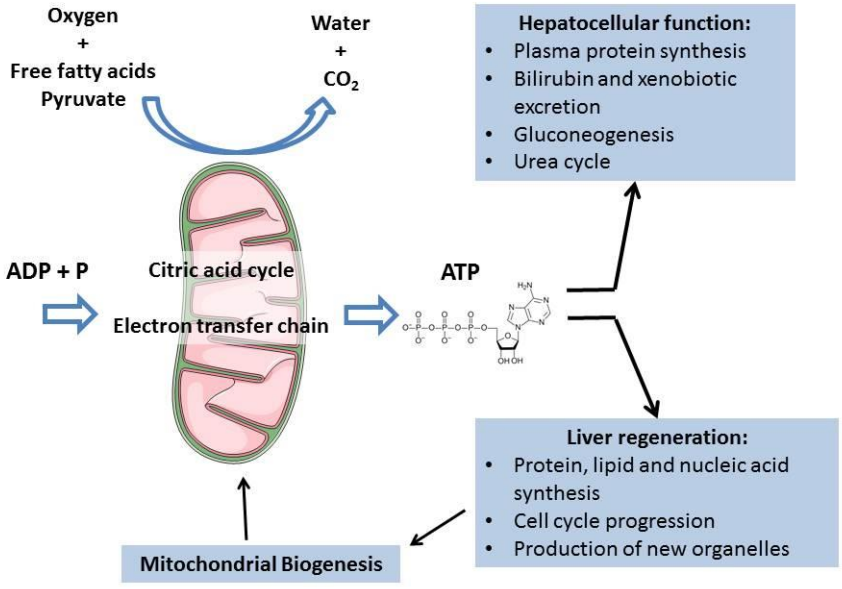
3. Posthepatectomy Liver Failure – Evidence for disturbed bioenergetics in pathophysiology

Clinical success of hepatectomy depends on the liver's unique ability to regenerate. After major hepatectomy, the remnant liver must replace lost hepatocyte

mass, produce an acute-phase response and still carry the burden of maintaining acceptable hepatocellular function for whole body homeostasis. After the surgical loss of liver tissue, regeneration does not rely on the proliferation of a progenitor cell population, but on the replication of normally quiescent hepatocytes, cells that already have high metabolic demands [16]. The enormous amount of energy for these processes is supplied by the oxidative phosphorylation of fatty acids by mitochondria under aerobic conditions (Figure 1.2) [39,43]. However, if the energy demand exceeds the supply, liver regeneration can be severely hampered resulting in PHLF and even death.

Figure 1.2 - Metabolic demands on mitochondria after hepatectomy

After hepatectomy mitochondria, through two oxygen-dependent enzymatic chains, the tricarboxylic acid cycle and the electron transport chain, supply hepatocytes with increasing amounts of ATP needed to fuel biosynthesis of cell components and progression through cell cycle. In the meantime, hepatocellular function is dependent upon a constant ATP supply. In ideal conditions, hepatocyte function will not be severely hampered and should return to normal as soon as possible. In the clinical setting good posthepatectomy outcome is confirmed by a return to normal values of arterial lactate (reflecting gluconeogenesis), prothrombin time (reflecting adequate protein synthesis) and serum bilirubin (reflecting conjugation and excretion of bilirubin, as well as other endo- and xenobiotics). All these liver functions are endergonic, i.e. energy-dependent. Interestingly, mitochondrial biogenesis is, in itself, a needed step for adequate hepatocyte replication.

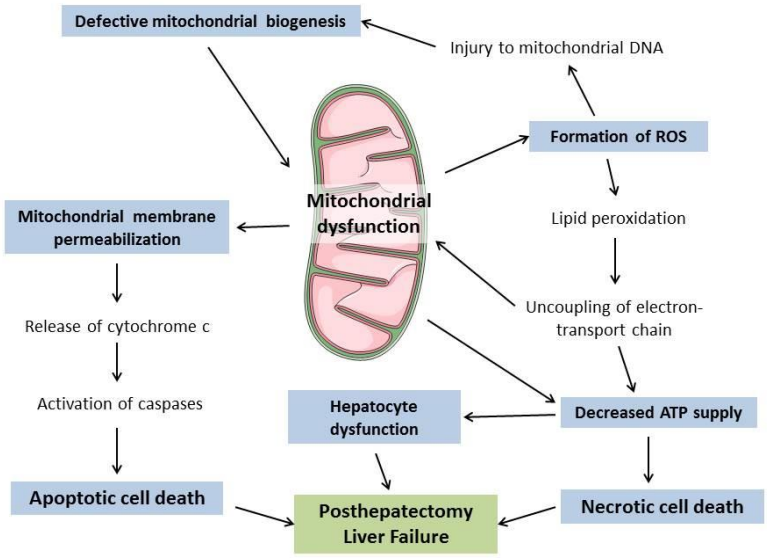


Disturbed bioenergetics is an important factor in several chronic liver diseases [54–56] and mitochondrial damage is involved in the pathophysiology of acetaminophen-induced acute liver failure [57] and possibly in other etiologies [58].

Mitochondrial dysfunction is characterized by decreased cellular ATP stores, compromised cellular processes and viability. Also, uncoupling of oxidative phosphorylation leads to increased production of reactive oxygen species (ROS), which can severely damage both mitochondrial DNA and inner membrane lipids, leading to decrease mitochondrial biogenesis and further uncoupling, respectively. Furthermore, mitochondrial membrane permeabilization releases cytochrome c in the cytoplasm and activates the caspase-mediated pathway of apoptosis, further compromising liver function (Figure 1.3). Finally, the delicate equilibrium of the cellular mitochondrial pool is maintained by the balance of formation of new and degradation of damaged mitochondria, by biogenesis and mitophagy, respectively, and if disturbed can further compromise the energetic status of the cell.

Figure 1.3 - A look into the mechanisms of mitochondrial dysfunction in Posthepatectomy Liver Failure

Uncoupling of oxidative phosphorylation causes increased production of reactive oxygen species (ROS) and decreased ATP production. ROS cause direct damage to mitochondrial DNA, further compromising mitochondrial biogenesis, while peroxidation of mitochondrial membrane phospholipids decreases energy efficiency. Ultimately, decreased ATP supply will hamper hepatocyte function and, if severe, lead to necrotic cell death through the loss of osmotic gradient because of failure of ATP-dependent ionic pumps. Finally, mitochondrial membrane permeabilization releases cytochrome c in the cytoplasm and activates the caspase-mediated pathway of apoptosis. The net result is a decrease in functioning hepatocyte mass and a derangement in hepatocellular function, clinically recognized as Posthepatectomy Liver Failure.



Clinical evidence for a role of bioenergetics derangement in posthepatectomy liver dysfunction is mostly indirect. The Arterial Ketone Body Ratio (AKBR), acetoacetate to 3-hydroxybutyrate ratio, is a marker of mitochondrial redox state. When there is impairment of the mitochondrial respiratory chain, NADH cannot be recycled to its reduced form (NAD+). In these conditions, higher quantities of 3-hydroxybutyrate are formed, decreasing the AKBR. Ukikusa et al studied the AKBR in a rabbit model of 70% hepatectomy and concluded that the early drop in AKBR was associated with a decrease in energy charge of the remnant liver [59]. These findings were corroborated in the clinical setting, with several studies demonstrating that an AKBR under 0.4 after hepatectomy was associated with decreased survival [60,61].

As the energy requirements for liver regeneration and function depend upon an efficient oxidative phosphorylation, the hepatic venous oxygen saturation could reflect the oxygen supply and demand of the liver parenchyma, and thus its energy charge [51]. The clinical evidence for this has been put forward by Kainuma et al [62]. The authors demonstrated that sustained decreases in intraoperative hepatic venous haemoglobin oxygen saturation were significantly correlated with peak postoperative aminotransferases, risk of PHLF and death.

Correlation of mitochondrial derangement with clinical outcome has been studied by Ozawa et al in a cohort of patients undergoing miscellaneous surgical procedures. Using liver biopsies taken during surgery, the authors found that patients with more pronounced changes in mitochondrial cytochrome activity were more likely to present with previous liver dysfunction, and also more prone to postoperative morbidity and mortality [63]. Later, the same author, with others, correlated the AKBR and postoperative complications after hepatectomy, with redox activity in liver samples [64]. However, more recently, another study failed to demonstrate a correlation between hepatic pedicle clamping, peak aminotransferases and mitochondrial respiration in liver resection [65].

We have recently demonstrated a direct relationship between mitochondrial bioenergetics and the postoperative outcome of liver resection [19]. By measuring mitochondrial membrane potential and oxygen consumption in two liver biopsies performed during liver resection (one at the beginning of the resection and the other just

at the end) in a cohort of 30 patients, we have demonstrated that depressed oxidative phosphorylation correlated with worse postoperative international normalized ratio (INR) and bilirubin, reflecting decreased liver synthetic and excretory function, respectively. Furthermore, depressed mitochondrial function was associated with increased risk of PHLF and was an independent risk factor for liver-specific morbidity. These data are further detailed in **Chapter II** – "*Mitochondrial Bioenergetics, Hepatic Pedicle Clamping and Posthepatectomy Liver Dysfunction*"**.**

Bioenergetics dysfunction after hepatectomy is probably multifactorial. First, transient deterioration of mitochondrial function has been proved to occur after hepatectomy in an animal model [66]. On the other hand, hepatectomy is often performed with Hepatic Pedicle Clamping (HPC) or Pringle manoeuvre [67,68], which is known to decrease mitochondrial function experimentally [69,70] (Figure 1.4). Clinical evidence for this has been indirect, with one electron microscopy study of intraoperative biopsies demonstrating that hepatectomy with HPC can cause mitochondrial swelling [71]. We sought to investigate on this matter and have found a significant correlation between longer HPC time and worse mitochondrial depolarization and lag phase [19]. Again the reader is referred to Chapter II for presentation of original clinical and experimental data on this subject.

Moreover, other mechanisms could be at play, including the hemodynamic changes after major hepatectomy. Since portal blood flow is dependent upon the splanchnic bed, after extended hepatectomy the reduced liver mass is exposed to an increased portal vein pressure (PVP). This causes increase in shear stress and induces nitric oxide production, which is recognized as an important stimulus to liver regeneration [72]. Paradoxically, excessive portal pressure can also be deleterious and contribute to PHLF, both in non-cirrhotic and cirrhotic patients, especially when PVP exceeds 20 mmHg [73,74]. The clinical presentation of disturbed synthetic function, ascites and increased risk of sepsis recapitulates the "small-for-size" syndrome (SFSS) of the transplant setting, occurring with the transplantation of a reduced size liver graft. The pathologic changes found in SFSS are portal vein endothelial denudation, centrilobular microvesicular steatosis and cholestasis, hepatocyte ballooning and ischemia [75]. More recently, the emphasis has been placed not on the volume of the

liver remnant, but on the exaggerated portal hyperflow, leading to a proposed renaming of the syndrome as “small for size and flow syndrome” [76].

However, shear stress is not the only mechanism thought to be involved. There is a physiologic mechanisms regulating total liver flow, the Hepatic Arterial Buffer Response (HABR), consisting of a reciprocal regulation of the hepatic artery flow by the portal venous inflow. When portal blood flow decreases, arterial dilation of the intrahepatic arterial bed compensates, maintaining constant hepatic blood flow. Conversely, portal hyperperfusion leads to a reduction in hepatic arterial blood flow [75,77,78]. The HABR is probably mediated by the washout of adenosine in the space of Mall by the increased portal flow, causing arterial vasoconstriction [79]. Other putative mediators of the HABR are nitric oxide, carbon monoxide and hydrogen sulphide [80]. Increase in blood norepinephrine concentrations has also been proposed as an underlying mechanism of hepatic artery vasoconstriction [81] but adrenergic blockade does not seem to reverse this phenomenon [82].

Since portal blood is poor in oxygen, increase in portal blood flow and decrease in arterial blood flow would lead to a state of parenchymal hypoxia, decreasing hepatic oxygen extraction and causing mitochondrial dysfunction and bioenergetics failure of the liver remnant. As such, we theorize that the deleterious effect of excessive portal pressure after hepatectomy is, at least in part, mediated by a decrease in hepatic oxygen extraction, which in turn leads to deficient ATP synthesis and bioenergetic failure (Figure 1.4). Although a small animal model demonstrated that extended hepatectomy was associated with decreased hepatic oxygenation and reduced mitochondrial oxidative phosphorylation, no decrease in hepatic artery flow was observed [83]. Nonetheless, this has not been the case in one larger animal model, where increase in portal vein flow and corresponding decrease in arterial blood flow were proportional to the extent of parenchymal resection [84].

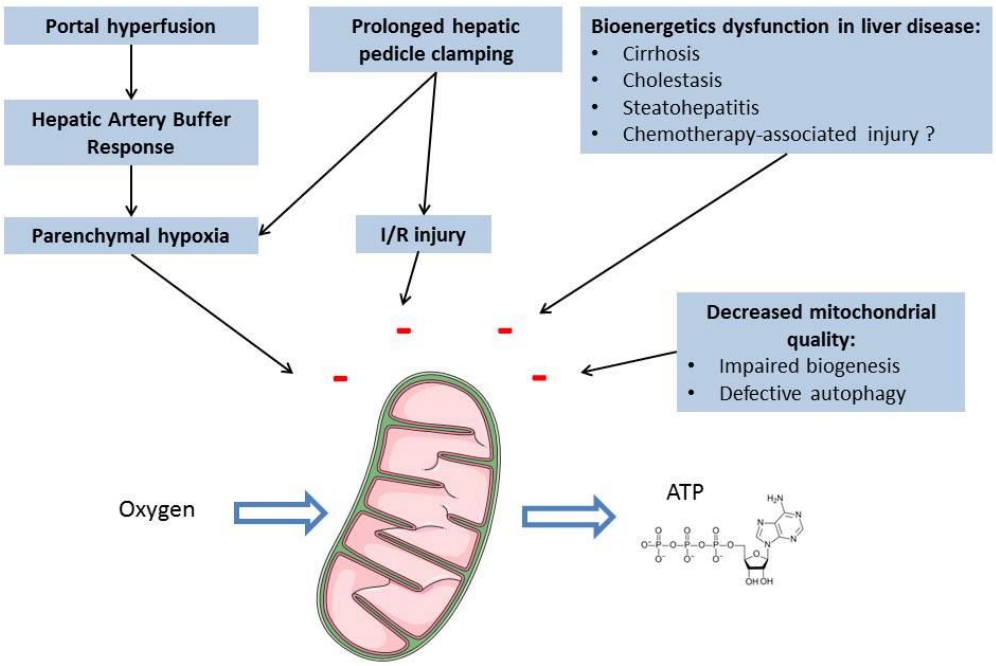
Although appealing in theory, the decreased hepatic artery flow and subsequent oxygen and energy deprivation of the liver remnant occurring in the small-for-size setting is probably not the only factor involved in the pathophysiology of PHLF. Apart from the deleterious effect of excessive shear stress, other mechanisms could be at play, such as disorganized replication of hepatocytes and sinusoidal endothelial cells [85].

The haphazard regenerative process is not accompanied by an efficient vascular network, potentially leading, early in the process, to an impaired oxygen supply to the rapidly dividing and metabolically active liver cells.

Bioenergetics dysfunction could potentially be even more relevant in patients with pre-existing mitochondrial impairment, such as chronic liver disease. In the following section we will briefly review the experimental and clinical evidence for impaired bioenergetics in the pathophysiology of PHLF in the setting of abnormal liver parenchyma.

Figure 1.4 - Pathophysiology of bioenergetics dysfunction in clinical liver surgery

Energetic efficiency can be compromised after hepatectomy and several different mechanisms are at play. After extended hepatectomy, a state of portal hyperperfusion of the liver remnant ensues. This is known as the "small-for-size" or "small-for-flow" syndrome. Portal hyperperfusion causes a reduction in hepatic arterial blood flow and oxygenation of the liver parenchyma, in a process mediated by the Hepatic Artery Buffer Response (HABR). Hepatic pedicle clamping, if used and prolonged in time, also contributes to parenchymal hypoxia and causes ischemia-reperfusion injury (IRI), which is known to cause mitochondrial dysfunction. Also, there is evidence for disturbed bioenergetics in several liver diseases, namely cirrhosis, biliary obstruction and steatohepatitis, making hepatectomy more risky in these settings. Finally, defective mitochondrial biogenesis and autophagy can decrease quality of liver mitochondria, decreasing energy yield and increasing production of reactive oxygen species (see text for details).



4. Hepatectomy in Chronic Liver Injury: Mitochondrial dysfunction as a key factor in the Pathophysiology of Posthepatectomy Liver Failure

Mitochondrial dysfunction has been recently linked to the pathogenesis of many acute and chronic liver diseases [54–56,86,87]. And while liver resection is ideally performed in patients with intact liver function, some candidates for hepatectomy present with chronic liver injury, which significantly increases the risk of postoperative liver dysfunction. The obesity epidemic has led to an increased prevalence of non-alcoholic fatty liver disease (NAFLD), making liver steatosis the main chronic liver disease in developed countries [88]. Hepatocellular carcinoma (HCC) usually arises in the setting of cirrhosis, and non-alcoholic steatohepatitis (NASH) is increasingly recognized as an important risk factor [89]. Moreover, some patients with colorectal cancer liver metastases, the main indication for hepatectomy in most countries, undergo multiple cycles of hepatotoxic chemotherapy, increasing the risk of major morbidity [90]. And finally, extended resections for hilar cholangiocarcinoma, are usually performed in the presence of biliary obstruction [5].

In the aforementioned conditions there is some degree of hepatic mitochondrial dysfunction, possibly aggravated in the postresection period. Thus it is no surprise that hepatectomy is fraught with an increased incidence of postoperative liver failure, mandating a correct preoperative stratification of surgical risk. In this section we will elaborate on the evidence for disturbed mitochondrial bioenergetics in the pathophysiology of PHLF in the setting of diseased liver parenchyma, hopefully aiding in the reduction of morbidity and mortality of liver resection in patients with chronic liver disease.

4.1 Steatosis and steatohepatitis

Steatosis and steatohepatitis are increasingly recognized as a significant public health problems, associated with the obesity and diabetes epidemics [88]. There are two distinct forms of non-alcoholic fatty liver disease (NAFLD): a more benign form, simple steatosis; and a more progressive, severe form - non-alcoholic steatohepatitis (NASH) often leading to cirrhosis, liver failure and hepatocellular carcinoma. In fact, in

developed countries NASH has supplanted other liver diseases as the main risk factor for HCC [89].

Mitochondrial dysfunction is a hallmark of NAFLD and this link has been extensively reviewed elsewhere [31,91–93]. The progression of simple fatty liver to florid steatohepatitis, fibrosis and cirrhosis is often viewed as two-hit process. In the first stage, caloric overload and insulin resistance cause intracellular accumulation of triglycerides in hepatocytes. And in the second stage mitochondrial dysfunction ensues, causing bioenergetics impairment, increased oxidative stress, inflammation and cellular death.

Mitochondrial dysfunction in NAFLD is multifactorial and includes impairment of oxidative phosphorylation and reduced ATP synthesis, loss of Adenine Nucleotide Translocator (ANT) in the mitochondrial outer membrane, increase in Uncoupling protein 2 (UCP-2), depletion of mitochondrial DNA and decreased expression of important transcription coactivators, like peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) [31]. More recently, epigenetic changes such as higher levels of methylation of mitochondrial DNA in NASH patients have been found to correlate with NAFLD activity score [94]. The progression of NAFLD to NASH is also associated with an increase in oxidative and nitrosative stress and reduction of antioxidant defences, like reduced mitochondrial glutathione (GSH) [31,95].

Other key pathophysiologic processes involved in the progression of fatty liver to NASH are inflammation and cell death. Oxidative damage to the hepatocyte gives rise to a pro-inflammatory state, further enhanced by systemic inflammation witnessed in metabolic syndrome [96]. This leads to neutrophil chemotaxis and amplification of the inflammation cascade. Tumor necrosis factor-α (TNF-α) is produced by Kupffer cells and can stimulate constitutively expressed receptors in the hepatocyte membrane, like Tumor necrosis factor-related apoptosis-inducing ligand receptor 1 and 2 (TRAIL-R1 and TRAIL-R2) [33]. Activation of these receptors leads to activation of caspase-8 and initiation of cell death. Here too, mitochondrial intervention is of paramount importance as hepatocytes require mitochondrial amplification of apoptotic response [97].

Studies of mitochondrial function in intraoperative liver biopsies have validated the importance of mitochondrial dysfunction in steatosis, such as lower respiratory

control ratio and lower expression of NRF-1 and mitochondrial transcription factor A (TFAM) [98], and lower baseline mitochondrial membrane potential and respiratory control ratio [19]. And interestingly, experimental studies have demonstrated that reversal of the metabolic derangements of NAFLD either by bariatric surgery [99] or by pharmacological therapy [100,101], can lead to an improvement in hepatic mitochondrial function.

Steatosis *per se* has been associated with impaired liver regeneration in experimental models. In an animal model of choline/methionine deficient diet, Veteläinen et al describe impaired liver regeneration after 70% hepatectomy, both in mild and in severe steatosis [102,103]. However, these results were not replicated in western-diet models of steatosis [104,105]. Nonetheless, since liver resection is often performed with inflow occlusion, fatty livers are more susceptible to IRI due to microcirculatory disturbances, Kupffer cell dysfunction, increased leukocyte adhesiveness, ATP depletion and mitochondrial failure [106–108]. In fact, Cauchy et al report that inflow clamping was an independent factor for major morbidity in patients with metabolic syndrome [109].

Nevertheless, the clinical evidence for an increased risk of PHLF and complications in steatotic livers is conflicting. There is even a suggestion for improvement in long-term results, with one retrospective study demonstrating increased survival in patients with steatosis after resection of colorectal cancer liver metastases [110].

Multiple studies have been published on the role of simple steatosis on outcomes of liver surgery, both in the setting of resection and in living-donor liver transplantation [106]. In a meta-analysis of studies of liver resection for neoplasm and living-donor procurement, Meijer et al concluded that patients with mild steatosis (< 30%) had increased morbidity, while moderate and severe steatosis (> 30%) were associated with increased morbidity and mortality [111]. However, most complications in patients with mild steatosis are infective complications [112] and some studies do not demonstrate an increase either in major morbidity or mortality [113–115].

Although controversial data emerges on the effect of milder degrees of steatosis on postoperative complications, most studies agree that severe steatosis and, in

particular, steatohepatitis are associated with a high risk of postoperative morbidity [116]. In a cohort of patients undergoing extended hepatectomy, Neal et al described that NASH was an independent risk factor for major morbidity and PHLF [117]. However, simple steatosis was not associated with worse outcome, on univariate and multivariate analysis. Interestingly, the only factor associated with increased risk of mortality on multivariate analysis in this study was diabetes mellitus. Insulin-treated diabetes was also found to significantly increase overall morbidity in another cohort of patients [118]. A case-control study by Reddy et al [114] demonstrated that steatohepatitis, but not simple steatosis, was associated with increased risk of overall and hepatic-related morbidity. Interestingly, an original clinical study from our group found that steatosis and steatohepatitis were associated with decreased morbidity [115] (see also **Chapter III** – "*Chemotherapy Associated Liver Injury and Posthepatectomy Morbidity: Possible contribution of Mitochondrial Dysfunction to the Pathogenesis of Sinusoidal Obstruction Syndrome*"). A non-exhaustive review of relevant clinical studies is detailed in Table 1.1.

Although the consequences of steatosis on clinical outcome after hepatectomy are diverse, the role of mitochondrial dysfunction in fatty liver disease is unequivocal. This, together with the increased susceptibility of fatty livers to IRI, could explain the increased morbidity and mortality of liver resection in patients with the most severe forms of NAFLD. Given the escalating epidemics of obesity, diabetes mellitus and metabolic syndrome, together with the expanding indications for liver resection, the spotlight is on understanding the mechanisms of bioenergetics dysfunction in steatosis, thus enhancing the safety of liver resection.

Table 1.1 - Summary of relevant clinical studies on the impact of steatosis and steatohepatitis on postoperative outcome after hepatectomy

Colorectal cancer liver metastases (CRLM); Hazard ratio (HR); Hepatocellular carcinoma (HCC); Non-alcoholic steatohepatitis (NASH); Odds ratio (OR); Posthepatectomy Liver Failure (PHLF); Relative risk (RR)

Author / Year (Ref.)	Study population	Setting	Conclusions
Behrns/ 1998 [119]	N = 135 • 56 mild steatosis • 7 moderate / severe steatosis	Mixed indications Resection of ≥ 4 Couinaud's segments No standardized definition of PHLF	Increased risk of morbidity, liver failure and mortality in moderate/ severe steatosis Increased postoperative bilirubin and aminotransferases
Neal / 2012 [117]	N = 103 • NASH 8.7% • Steatosis 41.7% (of which moderate / severe in 4.9%)	Extended hepatectomy (right trisectionectomy) for mixed indications (HCC, CRLM, other)	NASH independently associated with PHLF (HR=12.01; p=0.007) and major morbidity (HR 6.02; p=0.028) Steatosis was not associated with adverse outcome
Reddy / 2012 [114]	N = 348 102 patients with steatohepatitis and 72 patients with >33% steatosis vs. Matching controls	Case-control study Mixed indications (but cirrhosis and cholestasis were excluded) Mostly major or extended hepatectomy	Steatohepatitis was associated with increased hepatic-related morbidity (OR=2.7; p=0.016) but not PHLF or mortality Steatosis was not associated with adverse outcome
Kooby/ 2003 [112]	N = 223 pts with mild (<30%) steatosis N = 102 pts with marked (>30%) steatosis N = 160 matched controls	Case-control study Mixed indications (cirrhotic patients excluded)	Steatosis was associated with increased risk of morbidity, particularly infections Higher risk with marked steatosis (RR=3.04; p<0.01) No association with postoperative liver dysfunction or mortality
McCormack/ 2007 [113]	N = 58 patients with steatosis matched to 58 controls	Case-control study Major hepatectomy Mixed indications	Steatosis increased major morbidity (27% to 6.9%; p=0.001) Non statistically significant increase in PHLF by 50-50 criteria (8.6% vs. 1.7% in lean controls) Steatosis as an independent risk factor for postoperative morbidity, but not for mortality
Gomez/ 2007 [120]	N = 386 patients • Mild steatosis 122 • Moderate 60 • Severe 12 N = 193 controls	Colorectal cancer liver metastases 33.3% minor hepatectomies 66.6% major or extended hepatectomies	Severity of steatosis was identified as an independent risk factor for postoperative morbidity No increase in PHLF was described
Our previous report Martins/ 2016 [115] ***a)***	N = 140 patients • Mild steatosis 17% • Moderate and severe steatosis 11% • Steatohepatitis 16%	Colorectal cancer liver metastases 62% minor hepatectomies 38% major hepatectomies	Steatosis reduced the risk of postoperative complications (10.6% vs. 28%, p=0.014) and major morbidity (6.4% vs. 19.4%, p=0.047) Moderate and severe steatosis related with the absence of postoperative complications (0% vs. 24.8%, p=0.019) Steatohepatitis reduced the incidence of overall morbidity (4.3% vs. 25.6%, p=0.016)

a) Results of an original clinical study contained in this Thesis and detailed in Chapter III – "*Chemotherapy-Associated Liver Injury and Posthepatectomy Morbidity: Possible contribution of Mitochondrial Dysfunction to the Pathogenesis of Sinusoidal Obstruction Syndrome*"

4.2 Cirrhosis

Cirrhosis is the end-stage of infectious, immune, toxic or metabolic insults to the liver. It is characterized by decreased hepatocellular function and portal hypertension [121]. Hepatocellular carcinoma is a frequent complication and resection is one of the few curative treatments [4]. However, liver resection in cirrhosis is only possible in patients with preserved liver function and even then at the cost of a high risk of PHLF [122]. Moreover, cirrhotic livers are less tolerant to hepatic pedicle clamping, making liver resection in these circumstances more demanding [123].

The decreased regenerative response of cirrhotic livers has been previously noticed [124]. Among the contributing mechanisms, bioenergetics failure definitely plays a key role. Although other factors, including the microvascular distortion of the liver parenchyma act synergistically to decrease oxygen supply to liver cells [125], there is significant clinical and experimental evidence of deranged mitochondrial function in cirrhosis. Yang et al used a rodent model of chemically-induced cirrhosis to study the mitochondrial respiratory function and antioxidant capacity after hepatectomy [126]. After inducing cirrhosis with intra-peritoneal thioacetamide in Wistar rats, the authors performed 70% partial hepatectomy. After hepatectomy cirrhotic animals displayed decreased state 3 respiration, decreased activities of NADH-cytochrome c reductase and mitochondrial glutathione peroxidase, as well as decreased levels of mitochondrial glutathione, when compared with non-cirrhotic controls. Nishikawa et al [55], using a rat model of carbon tetrachloride and phenobarbital-induced cirrhosis, examined energy metabolism in isolated hepatocytes and concluded that in compensated cirrhosis maximal mitochondrial respiration is compromised and that ATP production was maintained by an increase in the glycolytic pathway. As cirrhosis progresses to decompensated form, failure of glycolytic pathway also occurs, leading to energetic failure. The authors confirmed these findings in gene-expression profile of human liver biopsy samples, as patients with compensated cirrhosis (Child A and B) had decreased expression of cytochrome oxidase 1 and 2 genes, with increased expression of genes for glycolytic enzymes. These enzymes were under-expressed in Child C cirrhosis, underlying the energetic failure that occurs in this premorbid state of decompensated liver disease. An interesting conclusion of this study is that mitochondrial function is hampered in early stages of cirrhosis, with glycolysis taking over most of the energy

production. As the glycolytic pathway is much less energy-efficient than oxidative phosphorylation, this could explain the decreased adaptation of compensated cirrhotic livers to resection and other acute stressors, such as infection [127]; as failure to increase ATP production would result in acute decompensation of liver function.

Deficient energetic recovery of the cirrhotic liver after hepatectomy has been demonstrated by Mann et al. [128]. Using ^{31}Phosphorus Magnetic Resonance Spectroscopy, the authors studied nine cirrhotic patients undergoing hepatectomy and compared with nine other patients with normal liver parenchyma. Cirrhotic patients experienced a sustained decrease in ATP to phosphate ratio and delayed regeneration versus patients with normal liver parenchyma.

Given the evidence for disordered energetics in cirrhosis, interventions aimed at improving energy status could be clinically relevant in prevention of hepatocellular dysfunction after liver resection. This will be further explored in this Chapter in Section 6. Improving liver regeneration – Hepatocyte energetics as a potential target for prevention of Postoperative Liver Failure.

4.3 Chemotherapy-induced liver injury

While hepatectomy is the gold standard of care for patients with colorectal cancer liver metastases, preoperative chemotherapy is increasingly used, not only in a conversion strategy but also in a true neoadjuvant intention [129,130]. The most commonly used drugs are 5-fluorouracil (5-FU), irinotecan and oxaliplatin. The cytotoxic agent 5-FU is a thymidylate synthase inhibitor and has been used for over 40 years in systemic therapy for advanced colorectal cancer. Irinotecan, or CPT-11, is a prodrug. It suffers transformation by carboxylesterase into SN-38, a potent inhibitor of topoisomerase-I. Oxaliplatin, a platinum derivative, forms DNA adducts and is directly cytotoxic [131]. Molecular targeted therapies, such as monoclonal antibodies, bevacizumab and cetuximab, have increased response and resectability rates [90]. Finally, in selected cases intra-arterial chemotherapy has demonstrated promising results in conversion to resectability [132].

However, chemotherapy is associated with hepatocellular injury and increased morbidity and even mortality after hepatectomy [116,133]. Although chemotherapy *per se* produces a decline in hepatocellular function (as measured by indocyanine green retention rate), independently of histologically-perceptible injury [134], chemotherapy-associated liver injury (CALI) usually occurs in distinct histologic patterns: steatosis and steatohepatitis (CASH); and sinusoidal obstruction syndrome (SOS).

Steatosis and steatohepatitis are usually associated with irinotecan-based chemotherapy and one study demonstrated an increase in morbidity and even mortality after liver resection [116]. Although the precise mechanism of hepatoxicity of irinotecan is unknown, it is thought to involve mitochondrial dysfunction [135]. Vauthey et al [116] reported a higher incidence of SH in patients treated with irinotecan-based chemotherapy, in particular with Body Mass Index over 25 kg/m^2. Patients with SH were more likely to suffer from posthepatectomy liver failure and had higher postoperative mortality. As the typical histologic injury of irinotecan-associated liver injury is steatohepatitis, we refer to the previous section (4.1 – Steatosis and steatohepatitis).

Sinusoidal obstruction syndrome (SOS) is another typical histologic pattern, and is usually associated with oxaliplatin chemotherapy [136]. It is characterized by severe sinusoidal dilation, sinusoidal endothelial cell necrosis and sloughing, causing extravasation of blood into the space of Disse and decreased sinusoidal flow [137]. One previous study from our group confirmed that SOS is an independent risk factor for postoperative morbidity [115] and usually courses with increased portal pressure, increased intraoperative bleeding, decreased tolerance to hepatic pedicle clamping and lower regenerative capacity [138–141]. Narita et al described increased morbidity in patients with histological evidence of chemotherapy injury during hepatectomy with prolonged hepatic pedicle clamping [139]. In 53 patients with chemotherapy-associated liver injury (mostly with oxilaplatin-related sinusoidal obstruction syndrome) undergoing major hepatectomy, the authors found a statistically significant increase in severe complications, namely sepsis and biloma, when hepatic pedicle clamping exceeded 30 minutes. No significant association was found, however, with increased incidence of postoperative liver failure, possibly due to the small study sample.

In yet another study, in a cohort of patients undergoing two-stage hepatectomy for colorectal cancer liver metastases, Narita et al again reported that sinusoidal obstruction syndrome was significantly associated with oxaliplatin-based chemotherapy, hampered volumetric increase of the remnant liver after first-stage hepatectomy, markedly depressed indocyanine green (ICG) clearance and caused a trend towards increased incidence of postoperative liver dysfunction [142]. Also, a blunted hypertrophic response was observed after portal vein embolization in patients with previous exposure to platinum-based chemotherapy [143].

Apart from DNA-directed effects, there is evidence to support that oxaliplatin is directly toxic to mitochondria, inhibiting oxidative phosphorylation in isolated liver mitochondria [144,145] and inducing mitochondrial-dependent apoptosis in cell cultures [146].

However, the lack of a valid animal model of oxaliplatin-induced toxicity has been a major drawback. Up until recently the only existing model of posthepatectomy liver dysfunction in toxic-induced SOS was the monocrotaline model [147]. The work of Robinson et al has shed new light into the pathophysiology of SOS. The authors validated an animal model of oxaliplatin-induced SOS in C57Bl/6 mice and found increase in oxidative stress in the injured parenchyma [148].

Although there is still no definitive link between mitochondrial toxicity and the development of SOS, we postulate that mitochondrial homeostasis could be severely hampered in chemotherapy-associated liver injury, thus leading to an increased susceptibility to postoperative liver dysfunction. Further investigation into this field is of paramount importance and has thus been the subject of investigation in this Thesis. In Chapter III original results from clinical and experimental works are presented.

4.4 Chronic biliary obstruction

Extended hepatectomy with *en bloc* bile duct resection is potentially curative for hilar cholangiocarcinoma [5,149]. However, liver resection under biliary obstruction is fraught with an extremely high incidence of postoperative liver dysfunction, with significant mortality. In the experience of a high-volume centre, Yokoyama et al report

as much as 38% incidence of moderate and severe PHLF in patients undergoing major or extended hepatectomy for biliary cancer, with severe PHLF associated with a 48% mortality rate [150]. Thus, preoperative biliary decompression is recommended to decrease the risk of PHLF and improve results in patients with high malignant biliary obstruction [151] and most centres nowadays include it in the preoperative algorithm for hilar cholangiocarcinoma, especially when there is a smaller future liver remnant (FLR) [5,149,152–154]. However, there is uncertainty as to what is the defined upper threshold of serum bilirubin mandating preoperative decompression; with cut-off values ranging from 2 to 10 mg/dL in most series [5,149]. Chronic cholestasis induces liver dysfunction through several mechanisms, including mitochondrial toxicity [155]. Conversely, biliary decompression results in an improvement in energy status of the liver. This is supported by clinical and experimental evidence which we will now summarily review.

Bile acids are directly toxic to mitochondria, causing a significant reduction of membrane potential, state 3 respiration and Respiratory Control Ratio, as well as increased susceptibility to Mitochondrial Permeability Transition (MPT) [156,157]. Mitochondrial biogenesis is also reduced by chronic biliary obstruction, as demonstrated by Arduini et al. In a rat model of bile duct ligation (BDL) there was a decrease in RCR and membrane potential, decreased expression of TFAM and depletion of mitochondrial DNA [158]. Surprisingly, in another animal model of segmental biliary obstruction, mitochondrial dysfunction was demonstrated not only in the obstructed lobe but also in the non-obstructed one [159].

Partial reversal of physiologic derangements by biliary decompression was demonstrated in an experimental model. Krähenbühl et al explored mitochondrial homeostasis in a rat model of BDL followed by Roux-en-Y anastomosis four weeks later [160]. The authors demonstrated that oxidative phosphorylation and β-oxidation of fatty acids were inhibited during cholestasis. After relief of obstruction there was an improvement in activities of complexes I and III of the mitochondrial electron transport chain, whereas the activities of complexes II and IV remained impaired. Furthermore, β-oxidation of fatty acids also remained depressed. Finally, in an extremely interesting clinical study, Mann et al used ^{31}Phosphorus magnetic resonance spectroscopy to study liver energy status in 10 patients with malignant biliary obstruction [161]. After

endoscopic or percutaneous biliary drainage, the ATP/Pi ratio, a measure of energy status, significantly increased in one week time, reflecting the beneficial effect on the entire liver parenchyma.

In conclusion, part of the biologic rationale for preoperative biliary decompression in the management of jaundiced patients undergoing major hepatectomy and bile duct resection can be related to improvement in mitochondrial function. If proved, further therapies aimed at mitochondrial bioenergetics could lead to an enhanced energetic status of the remnant liver, thereby decreasing the inherently high risk of postoperative liver dysfunction. Ultimately, this could result in a significant improvement in the management of perihilar cholangiocarcinoma.

5. Novel markers of mitochondrial dysfunction – Earlier diagnosis of Posthepatectomy Liver Failure

With the aim of standardizing the diagnosis and reporting of PHLF, a consensus definition was established in 2010 [162]. It defined PHLF as a postoperatively acquired deterioration in liver function, consisting of any increase of bilirubin and International Normalized Ratio (INR) on or beyond the fifth postoperative day. Serum bilirubin and INR reflect two major hepatocyte functions: excretory function (bilirubin uptake, conjugation and excretion); and synthetic function (namely of plasma clotting factors II, VII, IX and X) [163,164]. Usually, after uneventful hepatectomy these two parameters are within normal range by the fifth postoperative day, reflecting appropriate liver regeneration [11] (Figure 1.2). This consensus definition also defined clinical severity in three grades, according to the intensity of medical support required and the coexistence of other organ dysfunctions.

Another clinically used definition of PHLF using biochemical criteria is the “50-50 criteria” defined by Balzan et al. In 803 consecutive patients that underwent liver resection, the finding of bilirubin over 50 µmol/L and prothrombin time under 50% on postoperative day 5 was associated, after multivariate analysis, with increased postoperative mortality [165]. Different thresholds have been suggested in specific

subpopulations of surgical patients, such as peri-hilar cholangiocarcinoma [150] and cirrhotic patients with hepatocellular carcinoma [166].

Although straightforward to use and solidly placed in the clinical routine, these definitions are only valid on or beyond the fifth postoperative day, meaning that earlier liver dysfunction could be underdiagnosed. Furthermore, their use can be compromised in patients with previous biliary obstruction, who usually have high preoperative plasma bilirubin levels, while plasma transfusion can significantly change postoperative INR levels. Thus, the possibility of predicting liver dysfunction at an earlier time point is attractive, since it would allow more intensive treatment.

Since mitochondrial derangement likely occurs before the development of full-blown liver dysfunction [19], the resulting bioenergetics failure of the remnant liver can hamper liver regeneration [43]. Thus, the discovery of non-invasive serum markers of bioenergetics dysfunction is an attractive way for earlier detection of Posthepatectomy Liver Failure. These biomarkers can either be indirect metabolic markers of disturbed energetic status or can more directly reflect mitochondrial dysfunction.

Arterial lactate, an established prognostic marker in paracetamol-induced acute liver failure [167] and critical illness [168], can reflect the energy status of the hepatocyte. Lactate, produced by peripheral anaerobic glycolysis, is metabolized in the liver through the Cori cycle, whereby it is recycled to pyruvate and glucose in energy-consuming reactions partly occurring in the mitochondria [29]. In an animal model of PHLF, consisting of extended resection and remnant liver ischemia, Detry et al found significantly increased levels of lactate in the experimental group [169]. Watanabe et al proved that elevated arterial lactate on admission to the Intensive Care Unit after hepatectomy was independently associated with higher postoperative morbidity and mortality [170]; and more recently the value of early postoperative arterial lactate as an indicator of poor prognosis has been validated in a large prospective cohort [171]. Elevated arterial lactate not only reflects higher peripheral production (because of systemic hypoperfusion) but mostly the liver’s inability to adequately perform gluconeogenesis because of insufficient parenchyma or altered energy state. In fact, in a cohort of 14 patients, Theodorakis et al have demonstrated that, in conditions of ischemia-reperfusion, the liver becomes a net producer of lactate [172].

Serum phosphorus has also been scrutinized as a prognostic marker after hepatectomy. As liver regeneration usually requires an enormous supply of inorganic phosphate (for ATP synthesis, among other functions), a nadir in serum phosphate is not only frequent but also desirable after liver resection [173]. In fact, in a cohort of 719 patients undergoing major hepatectomy, Squires et al [174] have demonstrated that patients with profound hypophosphatemia (< 1.1 mg/dL) on postoperative day 2 experienced reduced incidence of PHLF and also major morbidity and 30-day mortality, when compared to patients with normal phosphatemia. However in this study, the majority of patients had parenteral phosphate supplementation. Furthermore, post resection drop in serum phosphate is also probably related to increased urinary loss [175], potentially compounding the use of phosphate as a reliable marker of acquired liver dysfunction.

The Arterial Ketone Body Ratio (AKBR), acetoacetate to β-hydroxybutyrate ratio, is a marker of mitochondrial redox state. In conditions of impairment of the mitochondrial electron transport chain, nicotinamide adenine dinucleotide (NADH) cannot be recycled to its oxidized form (NAD+), meaning that higher quantities of β-hydroxybutyrate are formed, decreasing the AKBR. Nakamura et al demonstrated that decreased hepatic perfusion during cardiopulmonary bypass in heart surgery was associated with a lower AKBR; however without deleterious consequences [176]. In the setting of liver resection Yamaguchi et al initially proposed that AKBR could predict survival after hepatectomy [60]. In this study, an AKBR under 0.4 was associated with increased arterial lactate and decreased survival. However, no association was sought with other markers of postoperative liver function. In another study, Yan et al also demonstrated that patients with AKBR under 0.4 had higher morbidity and mortality [177]. Higashi et al have specifically investigated the value of arterial ketone bodies measurement when compared with hepatic vein levels. They have found an excellent correlation between both measurements, suggesting that arterial ketone body levels reflect hepatic synthesis, rather than peripheral catabolism. In their cohort of 73 patients, the authors also found increased levels of β-hydroxybutyrate in patients with impaired liver function (worse ICG retention rate, higher postoperative bilirubin) and increased mortality [178]. Although promising, AKBR has not found its way into the clinical routine of most hepatobiliary centres.

Direct assessment of intra- and postoperative liver metabolism is possible with the use of microdialysis catheters, with serial measurements of intra-hepatic levels of lactate, pyruvate, glucose and glycerol. In the setting of liver transplantation microdialysis can assist in the early detection of severe ischemic injury [179,180]. At least two studies have reported the use of this technique in liver surgery. Isaksson et al, in 11 patients undergoing hepatectomy, reported increasing intra-hepatic levels of lactate, glucose and glycerol and increased lactate / pyruvate ratio during hepatic pedicle clamping [181]. And Windbladh et al explored the metabolic protection afforded by ischemic preconditioning in a randomized control trial involving 32 patients, demonstrating lower peri- and postoperative levels of intra-hepatic lactate in patients undergoing major hepatectomy [182]. Neither study, however, was designed to validate microdialysis as an early predictor of PHLF. In concept microdialysis is an extremely interesting approach, allowing bedside real-time monitoring of the lactate / pyruvate ratio, which accurately reflects energy metabolism. However, microdialysis is an invasive procedure as it requires the placement of an indwelling catheter on the liver parenchyma during hepatectomy. Further studies are needed in order to validate this technique for the early detection of PHLF.

Direct measurement of mitochondrial function in biopsies taken in the remnant liver at the end of hepatectomy could help predict PHLF. In personal work of our group we have previously demonstrated that mitochondrial lag phase (time to repolarize membrane potential after addition of ADP) was significantly longer in patients that went on to develop liver dysfunction, with a high specificity and sensitivity (area under the curve: 0.933; p=0.008) (see also Chapter II) [19]. However, this requires further confirmation in more studies. Furthermore, direct assessment of mitochondrial function in liver tissue, although feasible through a needle biopsy [183], is an invasive test and requires a dedicated bioenergetics laboratory for immediate sample processing. Ideally, mitochondrial dysfunction could be detected with non-invasive serum markers, with point of care tests that could find their way into routine clinical practice, from a “bench-to-bedside” approach.

Several circulating serum biomarkers of mitochondrial dysfunction, including ornithine carbamoyl transferase, alanine aminotransferase isoform 2 and cytochrome c, have emerged in the setting of drug-induced liver injury [184]. In particular,

acetaminophen-induced liver injury (AALF), one of the most common causes of acute liver failure in the developed world, has been the focus of attention. Mitochondrial toxicity is a hallmark event in its pathogenesis and not all intoxicated patients develop full-blown acute liver failure [57]. Thus, early and accurate identification of patients at risk is extremely important, as it can help in proper allocation of resources and timely transfer to higher levels of care, such as emergent referral for liver transplantation [185,186]. Several markers of mitochondrial dysfunction have been sought, namely mitochondrial DNA, circulating acylcarnitines and glutamate dehydrogenase (GLDH). The latter seems particularly promising, since Schomaker et al reported a high sensitivity and specificity of GLDH for diagnosis of toxic hepatocellular injury, with an area under the curve of 0.98 [187].

McGill et al reported on the use of serum mitochondrial biomarkers in a cohort of 69 patients with AALF [86]. They demonstrated that serum GLDH and serum levels of mitochondrial DNA (specifically for NADH dehydrogenase and cytochrome c oxidase subunit III) were higher in patients with AALF versus healthy controls. Furthermore, they demonstrated that these markers were significantly higher in non-survivors than in survivors. Using a Mitochondrial Biomarker Damage Index the authors report increased sensitivity and specificity in prediction of outcome.

In another work by the same group [185], circulating acylcarnitines were investigated in a cohort of patients with AALF. Acylcarnitines are carnitine-linked long chain fatty acids (e.g. palmitoyl-, linoleoyl- and oleoyl-), that are transported into the mitochondrial matrix for β-oxidation through facilitated diffusion by the carnitine-acetylcarnitine translocase (CACT). When β-oxidation is impaired these fatty acids derivatives accumulate in the cytosol and increased plasma levels are observed after cell necrosis. However, although promising in the rodent model, this marker did not show relevance in the clinical setting for AALF.

Albeit attractive, these mechanistic biomarkers were studied in the setting of acetaminophen toxicity, which is highly dependent on mitochondrial dysfunction. As such, extrapolation of these results for other mechanisms of liver injury, such as post-resection and post-ischemia-reperfusion injury must await confirmation of further studies.

In this Thesis we explored GLDH as a putative early diagnostic marker of PHLF. Although the original studies herein included were underpowered to prove its diagnostic accuracy, we nonetheless demonstrated important correlations of GLDH with other markers of postoperative liver dysfunction, such as serum bilirubin and arterial lactate (see Chapter III and **Chapter IV** – "*Bioenergetic Adaptations of the Liver in ALPPS: How Liver Regeneration Correlates with Mitochondrial Energy Status*" for more details). Hopefully, GLDH and other biomarkers of disturbed bioenergetics will find their way into clinical practice and allow for an earlier diagnosis of PHLF.

6. Improving liver regeneration – Hepatocyte energetics as a potential target for prevention of Postoperative Liver Failure

PHLF is associated with high mortality and current therapies are mostly supportive. The use of extracorporeal liver assist devices is an attractive option, including the Molecular Adsorbent Recirculating System (MARS) [188] but results have been largely disappointing and solid evidence is lacking in the particular population of patients with PHLF [189]. The treatment of PHLF has been the focus of extensive reviews and the reader is referred to them [8,10,190].

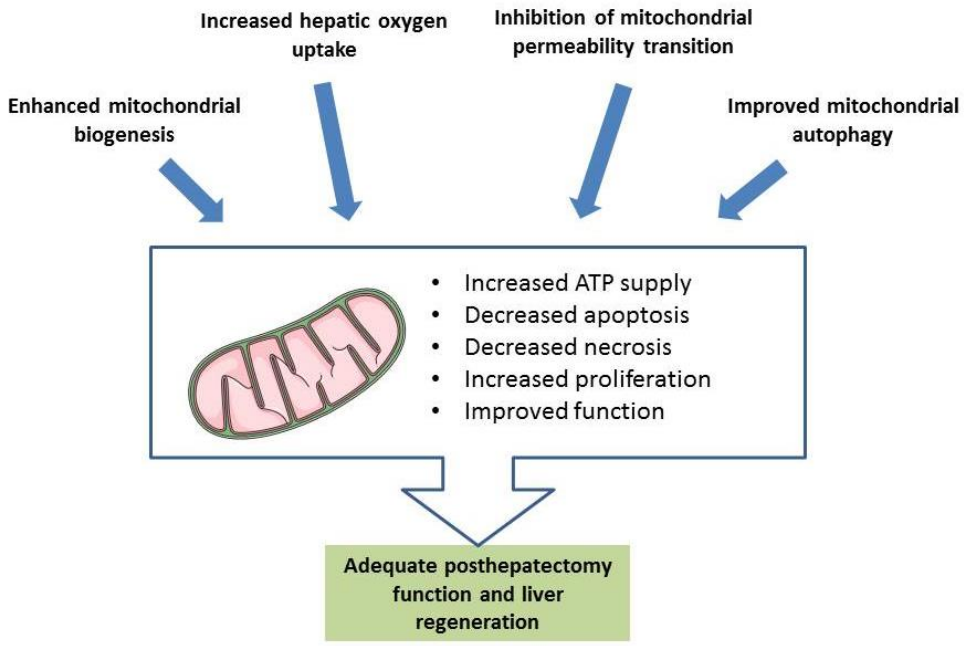
While in the previous sections we recapitulated the evidence for an important role of mitochondrial function in liver recovery following resection, much more appealing is the possibility of actually preventing PHLF by targeting mitochondrial homeostasis. Plausibly, energetic conditioning of liver cells could aid in the maintenance of adequate liver function and in the recovery of functioning liver mass after major hepatectomy. Several possible pathways could be explored, either isolated or in combination, include: improvement in mitochondrial pool by enhanced biogenesis and more efficient mitophagy; more efficient oxidative phosphorylation through increased parenchymal oxygenation; and decrease in mitochondrial permeability transition (Figure 1.5).

Because of the considerable overlapping of these pathways, we will now dwell on several therapeutic strategies, presenting them according to different methods: 1)

Pharmacological conditioning; 2) Strategies aiming at improving hepatocyte oxygenation; 3) Parenchymal modulation techniques; 4) Stem cell therapies.

Figure 1.5 - Mitochondrial-centered perspective on posthepatectomy outcome

Adequate recovery of liver parenchyma after resection depends upon a proper cellular energy homeostasis. Prevention of Posthepatectomy Liver Failure can be achieved by increasing hepatic oxygen uptake, such as intraoperative hemodynamic modulation, prevention of postoperative portal hyperperfusion, hyperbaric oxygen therapy or portal vein arterialization. Enhanced mitochondrial biogenesis is currently the focus of research in pharmacological preconditioning strategies and is likely involved in the mechanism of action of portal vein embolization and possibly two-stage hepatectomies. Improved mitochondrial autophagy and inhibition of the mitochondrial permeability transition are novel and yet clinically unexplored pathways that could potentially improve energy homeostasis in regenerating hepatocytes, enhancing ATP supply and decreasing production of ROS (see text for further details).



6.1 Pharmacological therapy

Mitochondrial-based therapies have been the focus of recent research. S-adenosyl-L-methionine (SAMe) is a methyl donor and a precursor of glutathione. Interestingly, Bailey et al demonstrated that SAMe improved mitochondrial respiration and oxidative phosphorylation, while decreasing mitochondrial ROS and damage to mitochondrial DNA in chronic ethanol exposure [191]. In another work, Brown et al report protection of SAMe against hepatic toxicity in an animal model of acetaminophen-induced liver injury [192]. And in an animal model of 70% hepatic

ischemia-reperfusion, Jeon et al demonstrated that pre-treatment with SAMe reduced peak AST, decreased lipid peroxidation, improved AKBR and maintained ATP levels [193]. Clinical evidence for use of SAMe as a pharmacological conditioning agent in liver resection has come from two randomized controlled trials [194,195]. Both studies demonstrated a decrease in peak postoperative aminotransferases and bilirubin, in particular with HPC over 15 minutes, in patients undergoing hepatectomy for hepatocellular carcinoma in cirrhosis. However, no mechanistic study was performed and we can only theorize that the protection afforded by SAMe is, at least in part, directly related to improvement in mitochondrial respiration and in oxidative phosphorylation. Further studies into the clinical applications of SAMe in liver surgery are needed, in particular from a mitochondrial-centred perspective.

Berberine, an isoquinoline alkaloid found in several plants used in traditional Chinese medicine, is another promising compound. Known for its antidiabetic properties, it has demonstrated beneficial effects on mitochondrial oxidative phosphorylation in fatty liver models, mostly through Sirtuin 3 mediated mechanism, possibly involving enhanced mitochondrial biogenesis, among other actions [100]. Other possible mitochondrial-centred positive effects include ethanol-induced liver toxicity and renal ischemia-reperfusion injury [196,197]. At least one clinical trial has proved its safety and efficacy in NAFLD [198], suggesting that berberine could be a valid alternative for energetic conditioning of liver cells before hepatectomy. Further studies are needed, however.

One of the most exciting candidate molecules for energetic conditioning of liver cells is the Augmenter of Liver Regeneration (ALR). Having been first isolated and purified by Labrecque et al [199], ALR is also known as Hepatic Stimulator Substance or Hepatopoietin, for its strong mitogenic effect, far surpassing other known hepatic mitogens such as Epidermal growth factor (EGF) and Transforming growth factor alpha (TGF-α). ALR is secreted in liver cells and acts on Kupffer cells and in autocrine and paracrine fashion on hepatocytes. It is also found in the intracellular compartment, namely in mitochondria, where it regulates oxidative phosphorylation and is essential for mitochondrial biogenesis and ATP synthesis [200]. The importance of ALR in liver regeneration seems to be dependent upon its effect on mitochondrial biogenesis, uphill of other two co-factors, TFAM and PGC-1α [21]. In fact, in this Thesis we describe an

increased expression of ALR RNA in the remnant liver of patients during the second stage of two-stage hepatectomies (see Chapter IV). In one experimental study, transfection of isolated hepatocytes with antisense oligonucleotide against ALR brought about energetic failure and cell death by apoptosis and necrosis [201], while transfection of ALR via adenovirus in rodent models of hepatic ischemia reperfusion and toxic injuries demonstrated improved oxidative phosphorylation, increased ATP production, decreased mitochondrial permeability transition, reduced cell death and improved survival [202,203]. Interestingly, delivery of ALR is not limited to gene transfection as at least two studies demonstrated that intraperitoneal injection was also effective in improving mitochondrial bioenergetics and biogenesis [204,205]. This ease of administration, not requiring ALR integration into the host genome, could open the possibility of expanding ALR therapy into the clinical arena for the treatment of acute liver failure of several etiologies, including PHLF.

Another potential therapeutic target is inhibition of the MPT. At least two experimental studies have demonstrated promising results with N-methyl-4-isoleucine cyclosporine (NIM811) in the setting of posthepatectomy liver failure and in small-for-size liver transplant [20,53]. The search for small molecule inhibitors of the MPT is ongoing and future advances are expected in the clinical arena in the near future [206,207].

Finally, induction of autophagy is yet another possible mechanism of hepatic energetic conditioning and a plausible therapeutic avenue to pursue with pharmacologic agents [37]. By the selective removal of damaged mitochondria, enhanced mitophagy is thought to increase the energetic efficiency of cells and decrease oxidative stress, thus improving liver regeneration and survival in a rodent model of 90% hepatectomy [208].

6.2 Improving hepatocyte oxygenation

Intraoperative increase of hepatic oxygen delivery and extraction could potentially prevent postoperative hepatocellular dysfunction by increasing the energy supply to the rapidly dividing and metabolically active liver parenchymal cells [62,209]. Since the use of inflow occlusion to minimize bleeding during liver resection causes

inevitable liver ischemia, diverse clamping techniques and strategies have been developed to minimize the impact on the liver parenchyma. This subject has been extensively detailed elsewhere [68,210–212]. In this review we will shed some light on novel or less explored approaches to increase parenchymal oxygenation. Several strategies aiming at this goal have been pursued, some of them with clinically relevant results.

Intraoperative hyperdynamic circulatory pharmacological manipulation with β–adrenergic agonists is an attractive option, as it increases hepatic arterial flow, portal vein oxygen saturation and hepatic oxygen delivery, with subsequent increase in liver lactate uptake [213]. In an extremely interesting prospective randomized trial with 30 compensated cirrhotic patients undergoing hepatectomy for HCC, Taurà et al demonstrated that low dose β-adrenergic agent dobutamine decreased portal vein resistance, increased hepatic blood flow and improved hepatic oxygen delivery, consumption and extraction. In the dobutamine-treated group this resulted in increased lactate clearance and decreased peak serum AST and peak postoperative bilirubin [214]. Although underpowered to prove a decrease in morbidity with the pharmacologic intervention, this study is fascinating as it conclusively demonstrates that enhancement of hepatic aerobic metabolism improves postoperative liver function.

Modulation of portal flow is another option, since extended hepatectomies result in portal overflow and subsequent arterial vasoconstriction, mediated by the Hepatic Arterial Buffer Response [79] (see Section 3. Posthepatectomy Liver Failure - Evidence for disturbed bioenergetics in pathophysiology). This relative liver hypoxia during the post-resection acute increase in metabolic demands could theoretically contribute to early liver remnant bioenergetics failure. Thus, reversal of excessive portal flow has been explored in order to prevent postoperative liver dysfunction. Several different animal models have explored surgical measures to decrease splanchnic blood flow, namely jejunal resection, splenectomy, splenic artery ligation and portal vein banding. Kawano et al, performing jejunectomy and 70% hepatectomy in dogs, reported improved hepatocellular function and decreased necrosis and apoptosis [215]. In a rodent model of 90% hepatectomy, splenectomy has been demonstrated to decrease portal vein flow, improve regeneration and decrease postoperative liver failure [216]; this effect is linked to an increase in hepatic arterial blood flow and hepatic oxygenation

and is likely mediated by the HABR [217,218]. Decreased mitochondrial swelling has also been described after splenectomy as a portal flow modulation strategy after 80% hepatectomy [219]. Splenic artery ligation has also been tested in experimental models, with encouraging results [220]. Portal vein banding is another promising method and one preclinical study in porcine model has reported improved postoperative liver function, however without demonstrating increased parenchymal oxygenation or improved hepatocellular metabolism [221]. These studies have been followed by clinical attempts to modulate portal blood flow as a strategy to improve liver regeneration, in the setting of partial liver transplantation [222,223] and extended hepatectomy (E. Vibert, personal communication)[224,225].

Interestingly, improved arterial flow could in fact be modulated independently of portal flow, as Kelly et al demonstrated in a porcine model of small-for-size liver transplant. Using intra-arterial adenosine perfusion starting on postoperative day one through a catheter placed in the gastroduodenal artery, the authors describe increasing arterial blood flow without modulating portal flow, with adenosine treated animals experiencing improved survival, decreased ascites, improved liver function and reduced necrosis score, versus controls [226]. If further studies confirm these findings, the aim of therapies could shift from reducing portal flow to increasing arterial flow, and hence parenchymal oxygenation, in PHLF and SFSS.

Given that the rationale for the aforementioned strategies is the increase in liver oxygen uptake, hyperbaric oxygen therapy (HBO) would seem a reasonable approach. Although there is both preclinical and clinical evidence to support its use, it has been largely unexplored. One experimental study has demonstrated improvement in liver regeneration and ATP content after 90% hepatectomy with 2 atmospheres 80% O_2 therapy [227]. Mitochondrial function was assessed in one animal model of 70% hepatectomy and although there was a decrease in RCR in the HBO-treated group, there was also an in improvement in liver regeneration rate, DNA content and proliferation index [228]. There is undoubtedly a beneficial effect in liver bioenergetics, as HBO can prevent deterioration of RCR, maintain mitochondrial membrane potential and sustain ATP synthesis in isolated mitochondria in experimental conditions of cold ischemia reperfusion injury [229]. The clinical evidence for the use of HBO in liver surgery has come mostly from the pediatric transplantation setting where it has shown benefits in

the management of hepatic artery thrombosis [230,231]. Apart from case reports demonstrating its anecdotal use in acute liver failure [232], including one case of PHLF [233], at least two prospective clinical trials have reported encouraging results on its use after hepatectomy. Ueno et al randomized 41 cirrhotic patients with hepatocellular carcinoma after hepatectomy complicated with major intraoperative blood loss to either HBO or standard management [234]. HBO-treated patients experienced lower postoperative arterial lactate and bilirubin, higher hepatic vein oxygen saturation and a trend towards improved hepatocellular function. However, in this study the definition of PHLF was not standardized so no conclusions can be drawn on this respect. Suehiro et al studied the effect of HBO on seven left liver living-donors and compared them to seven standard-treated equally-matched controls [235]. HBO-treated donors presented with better late postoperative liver function and increased liver remnant volume at four weeks. As such, HBO has proved safe and feasible, mandating the call for a randomized controlled trial on its use after major hepatectomy in patients at high risk of PHLF.

Inasmuch as increasing liver oxygen uptake could improve bioenergetics and hence prevent liver dysfunction, another potentially useful technique would be portal vein arterialization (PVA). In fact, PVA in animal models is known to increase portal venous flow and oxygenation, liver ATP content and survival after extended hepatectomy [236], with corresponding increase in liver regeneration rate and cell proliferation [237,238]. Although an appealing therapy in concept, its clinical use has mostly been reported for salvage therapy of a totally de-arterialized liver [239,240] and cannot, at the present moment, be proposed as a first-line therapy for prevention of PHLF.

6.3 Parenchymal modulation techniques

One of the most widely used strategies in prevention of PHLF is portal vein embolization (PVE), or portal vein ligation (PVL). First described in the English literature in 1986 [241], PVE is an Intervention Radiology technique that shunts portal blood away from the tumour-bearing parenchyma into the FLR, causing atrophy of the former and hypertrophy of the latter [242]. One randomized controlled trial demonstrated that PVE decreased the rate of postoperative complications, improved

liver function and shortened intensive care unit and total hospital stay in patients with chronic liver disease undergoing major hepatectomy [243]. There is experimental evidence for modulation of mitochondrial function in the biologic rationale of PVE, as there is an early increase in oxidative phosphorylation and respiration in the non-embolized lobe in a rabbit model [244]. Katoh et al have demonstrated an early and sustained increase in mitochondrial function in the non-embolized lobes in a rat model [245], possibly mediated by a marked increase in mitochondrial DNA and mRNA that occurs early after PVE [246]. More recently, the hypertrophic response to PVE was also associated with the Nuclear factor erythroid 2-related factor 2 (Nrf-2) transcription factor [247], a key inductor of mitochondrial biogenesis [34].

Two-stage hepatectomies, including the Associating Liver Partition and Portal Ligation for Staged Hepatectomy (ALPPS) procedure [248,249], have emerged in the armamentarium of liver surgeons. They use the same physiologic principle of PVE and PVL, namely diversion of portal flow to the remnant liver. In the case of the ALPPS procedure, parenchymal transection is also performed and is associated with a markedly increased liver hypertrophy [250]. This greatly enhanced volume increase is dependent upon an enhanced proliferative index, increase in hepatocyte cytoplasm and nuclear size [251]. Although the parenchymal response is similar to that observed with PVE, the hepatocyte morphology is more immature, with less organelles [252]. This inordinately rapid regenerative response probably requires important metabolic adaptations in order to sustain the increased energy requirements of rapidly dividing hepatocytes. However, the mechanisms underlying these adaptations in cellular bioenergetics are largely unknown and have also been the subject of interrogation in this Thesis. In Chapter IV we present evidence for several inter-stages metabolic changes, including enhanced bioenergetics and increased mitochondrial biogenesis in ALPPS, never previously reported in the scientific literature.

6.4 Stem cell therapy

Cell therapy has emerged as an important approach for end-stage liver disease, with both clinical and experimental studies reporting on the beneficial impact of

mesenchymal, embryonic and induced pluripotent stem cells on liver regeneration after toxic and surgical insults [253].

In part, the effect of stem-cell therapy could be mediated by an improvement in mitochondrial function, as there is an increase in mitochondrial number [254], decrease in mitochondrial-mediated apoptosis [255] and improved mitochondrial respiration [256]. In a very interesting study, Andrade et al have demonstrated that bone marrow mononuclear cells transplanted through the jugular vein 14 day after BDL in Wistar rats resulted in improved mitochondrial respiration (state 3 and RCR), decreased lipid peroxidation and decreased fibrogenesis [257]. Tautenhahn et al [258] studied the effect of mesenchymal stem cells on an animal model of PHLF. They found that the improvement in liver function, decreased apoptosis, increased regeneration and overall better metabolic profile occurred very early after therapy and were independent of stem cells' engraftment. As such, an interesting phenomenon could be taking place, as stem cells could potentially transfer mitochondria to liver cells, thus improving their metabolic capacity. The transfer of mitochondria is thought to occur through intercellular nanotubes [259] and direct intrasplenic injection of isolated mitochondria has also proved beneficial in experimental models of liver ischemia-reperfusion injury [260]. Although stem cell therapy for PHLF has been mostly experimental, some clinical trials have addressed the possibility of using CD133+ stem cells in the prevention of PHLF, in particular as adjuvant to portal vein embolization [261,262]. As further clinical studies are underway in cell therapy for liver disease, it would be interesting to evaluate the effect on mitochondrial function, in particular in the setting of acute liver failure and PHLF.

Conclusions and future prospects

Although the subject of bioenergetics in liver regeneration and clinical liver surgery has been explored in the past, recent clinical and experimental evidence has rekindled the interest in this field. With the increased prevalence of chronic liver disease and the expanding indications for hepatectomy, novel strategies to improve the results of liver surgery are paramount. Albeit a wondrous phenomenon, liver regeneration is

not inexhaustible and some patients still die from liver dysfunction in spite of optimal pre-, intra- and postoperative management, even in the most experienced hepatobiliary surgery centers. As liver physiology is evermore so taken to its limits, a profound knowledge of the factors involved in liver regeneration is desperately needed, including the energy source that fuels it. Mitochondria, the main energy suppliers of eukaryote cells and key players in cell death, find themselves at the heart of this process, and in the spotlight of biomedical research.

The clinical importance of bioenergetics in liver surgery is threefold. Firstly, bioenergetics status of the liver parenchyma could become an important prognostic marker of preoperative liver reserve, in the same way as volumetry or ICG retention rate [263,264]. This would be of particular relevance in cases of diseased liver parenchyma, whereby an accurate assessment of liver functional reserve is sometimes exceedingly difficult. Indeed, the search for precise functional assessment of the FLR is still ongoing, with an increasingly greater emphasis on dynamic or segmental function tests (kinetic growth rate, differential assessment of right versus left liver function by hepatobiliary scintigraphy or gadoxetic acid-enhanced magnetic resonance) rather than static or whole-liver function tests [265–268]. As both dynamic and static liver function tests ultimately depend on the assessment of endergonic, or ATP consuming reactions, measurement of energy status of liver cells could in itself be considered a liver function test [269]. In this regard, preoperative bioenergetics capacity would potentially translate into clinical practice as a significant measure of parenchymal tolerance to surgical insult.

Secondly, as the evidence for the key role of mitochondria in the pathophysiology of posthepatectomy liver failure unfolds, so does the possibility of using markers of mitochondrial dysfunction in the early detection of this dreaded complication gain even more relevance. In fact, several serum biomarkers are currently being explored in the setting of toxic-induced acute liver failure and could also prove their worth in the early diagnosis of PHLF. Furthermore, novel approaches like microdialysis could potentially aid in the early detection of the metabolic derangements that precede full-blown liver failure in high-risk patients.

Finally, as the possibility of direct interventions on liver energy homeostasis proves useful in improving clinical outcomes, a fascinating new field of mitochondrial-directed therapies emerges into the clinical arena. Several noteworthy therapeutic strategies developed to improve postoperative liver function have demonstrated to have an underlying rationale based on enhanced cellular bioenergetics. This is the case for preoperative biliary drainage, portal vein embolization and hyperbaric oxygen. Thus, novel approaches such as pharmacological induction of mitochondrial biogenesis and mitophagy, inhibition of the permeability transition, portal flow modulation or stem cell therapy could ultimately result in an improvement of cellular energetics and substantially decrease the morbidity and mortality of liver resection.

In conclusion, as Nagino et al brilliantly stated, "*the tolerable extent of radical resection depends on the capacity for regeneration of the remnant liver*" [46]. And by becoming able to improve energetic conditioning of the liver parenchyma we will safely expand the limits of hepatic surgery; potentially benefiting patients nowadays deemed inoperable due to a predicted insufficient liver remnant.

Chapter II - Mitochondrial Bioenergetics, Hepatic Pedicle Clamping and Posthepatectomy Liver Dysfunction

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I. Introduction

Hepatectomy, the only curative treatment for many patients with primary and secondary malignant neoplasms, ultimately relies on the liver's capacity to replace lost hepatocyte mass, while still producing an acute-phase response and maintaining adequate hepatocellular function [43]. All these processes require an enormous energy supply, which, if not met, can contribute to a derangement in liver function known as Posthepatectomy Liver Failure (PHLF), a severe and sometimes lethal complication of liver surgery [165].

Mitochondria are the powerhouses of eukaryote cells, hosting the most important energy-producing reactions: β-oxidation of fatty acids, the tricarboxylic acid cycle and the electron transport chain. The electron transport chain (ETC) is the key enzymatic machinery for production of adenosine triphosphate (ATP). In the ETC protons are moved from the mitochondrial matrix into the inter-membrane space, creating an electro-chemical gradient. The energy for the creation of this chemiosmotic gradient is provided by the sequential transport of electrons along the ETC complexes I to IV from two intermediate molecules, flavin adenine dinucleotide ($FADH_2$) and nicotinamide adenine dinucleotide (NADH), to molecular oxygen. The return of protons to the mitochondrial matrix is coupled to ATP synthesis by complex V (F_1F_0 ATP-synthase). The uncoupling of oxidative phosphorylation causes a decrease in energy production, and the resulting energetic failure causes cell death by necrosis. Apoptotic cell death can also occur because of induction of mitochondrial membrane permeability transition, releasing cytochrome c and activating the caspase-dependent pathway of apoptosis. Mitochondrial membrane potential can be measured experimentally and is, along with oxygen consumption and ATP content, an excellent indicator of cellular energy homeostasis [270,271].

Mitochondrial dysfunction is an important factor in the pathophysiology of many liver diseases [54–56,86,272]. Experimental evidence suggests a key role for bioenergetics in post-resection liver regeneration [52,66] and this approach has recently

regained interest [20–22]. In order to reduce blood loss, hepatectomy is often performed under inflow occlusion, or Hepatic Pedicle Clamping (HPC), causing ischemia-reperfusion injury [67,68,273,274]. Isolated HPC without hepatectomy is known to cause mitochondrial dysfunction experimentally [69]. However, the impact of hepatectomy with HPC on mitochondrial function in animal models is largely unknown. And furthermore, the precise role of mitochondrial dysfunction in clinical liver resection is yet to be investigated. Hence, the research questions underlying this Chapter were:

1. What is the effect of hepatectomy with hepatic pedicle clamping on liver mitochondrial function?
2. What is the clinical relevance of energetic status and mitochondrial function on the postoperative outcome, liver function, and morbidity of patients undergoing liver resection?
3. And as secondary endpoint, how are baseline mitochondrial oxidative phosphorylation and respiration influenced by preoperative status, in particular by age and co-morbidities?

In the attempt to answer these questions we designed an experimental study to assess the effect of major hepatectomy with intermittent hepatic pedicle clamping on mitochondrial oxidative phosphorylation, respiration and liver injury. A prospective, observational clinical study was also conducted to investigate the intraoperative changes in cellular bioenergetics in liver biopsies of patients undergoing hepatectomy; we then correlated these findings with pre-, intra- and postoperative variables, especially posthepatectomy liver function and morbidity.

II. Materials and Methods

A. Experimental study

1. Study animals

Animals, 12 weeks old male Wistar rats (*Rattus norvegicus*) weighing 267 – 323 g were purchased from Charles Rivers (Charles Rivers, France). Upon arrival animals were allowed to acclimatize and were housed in 12-hours light-dark cycles, with

controlled temperature and humidity, with unlimited access to food and water. The study protocol was approved by the Animal Ethics Committee of the Faculty of Medicine of the University of Coimbra and all animals received care according to institutional guidelines.

2. Surgical protocol

Surgical procedures were conducted under isoflurane anaesthesia, by the same operator, between 7:00 and 8:00 am. The experimental model of hepatectomy consisted of the resection of the median (ML) and left lateral lobes (LLL), comprising a 68% to 70% reduction, according to Martins et al [275]. A median laparotomy was performed and the liver mobilized by division of the triangular and hepatogastric ligaments. Ligation of the vasculo-biliary pedicles to the ML and LLL was performed with polyglactin 4/0 (Vicryl, Ethicon, USA). For hepatic pedicle clamping, the liver hilum was isolated just cranial to the duodenum and a microvascular bulldog clamp placed for two periods of 15 minutes, with a 5-minutes interval of reperfusion. Adequate inflow occlusion was confirmed by absence of visible pulsation in the hepatic artery distal to the clamp and by the onset of venous congestion and edema in the splanchnic territory.

Animals (n=35) were divided in four groups:

- **Group 1** or control group (n= 7), underwent sham laparotomy, isolation of hepatic pedicle and gentle liver manipulation, with the abdomen open during 35 minutes;
- **Group 2** (n= 14), underwent 70% hepatectomy; the hepatic pedicle was isolated but not clamped and the abdomen was left open for 35 minutes;
- **Group 3** (n= 7), underwent intermittent HPC (during 15 minutes, followed by 5 minutes reperfusion and 15 minutes clamping again) and gentle liver manipulation, but without hepatectomy;
- **Group 4** (n= 7), underwent 70% hepatectomy with intermittent HPC (during 15 minutes, followed by 5 minutes reperfusion and 15 minutes clamping again).

At the end of the procedure the abdomen was closed in two layers with polyglactin 4/0 (Vicryl, Ethicon, USA) in all groups; all operated animals were allowed to recover.

Animals were sacrificed at 12h, 24h, 48h and 72h after the surgical procedure, by craniocervical dissociation. Blood was collected from the inferior vena cava, and remnant liver (in groups 2 and 4) and whole liver (in groups 1 and 3), was weighed and removed.

3. Mitochondrial isolation

Mitochondria were isolated in homogenization medium containing 250 mM sucrose, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (pH 7.4), 0.5 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), and 0.1% fat-free bovine serum albumin (BSA), as previously described [69,276]. After homogenization of the minced blood-free hepatic tissue, the homogenate was centrifuged at 800 g for 10 min at 4º C. The supernatant was spun at 10,000 g for 10 min at 4º C to pellet mitochondria, which were re-suspended in a final washing medium. EGTA and bovine serum albumin were omitted from the final washing medium, adjusted at pH 7.4. Protein content was determined by the biuret method calibrated with bovine serum albumin.

4. Measurement of mitochondrial membrane potential

Mitochondrial membrane potential was estimated using an ion-selective electrode to measure the distribution of tetraphenylphosphonium (TPP^+). The voltage response of the TPP^+ electrode to log (TPP^+) was linear with a slope of 59 ± 1, in conformity with the Nernst equation. Reactions were carried out at 25º C, in a temperature-controlled water-jacketed chamber with magnetic stirring. Mitochondria (1 mg) were suspended in 1 ml of standard respiratory medium (130 mM sucrose, 50 mM KCl, 5 mM $MgCl_2$, 5mM KH_2PO_4, 50 µM ethylenediaminetetraacetic acid EDTA, and

5 mM HEPES [pH 7.4] and 2 µM rotenone supplemented with 3 µM TPP^+). A matrix volume of 1.1 µl/mg protein was assumed.

The measured parameters were: membrane potential (mV); depolarization (mV); lag phase (seconds); and repolarization (mV). Readings were recorded in duplicate.

5. Measurement of oxygen consumption

Oxygen consumption of isolated mitochondria was polarographically determinated with a Clark oxygen electrode (Oxygraph, Hansatech Instruments Ltd, UK) as described [69]. Mitochondria (1 mg) were suspended under constant stirring, at 25ºC, in 1.3 ml of standard respiratory medium (130 mM sucrose, 50 mM KCl, 5 mM MgCl2, 5mM KH_2PO_4, 50 µM EDTA, and 5 mM HEPES (pH 7.4) and 2 µM rotenone). State 3 respiration was induced by adding 200 nmol ADP. The oxygen consumption was also measured in the presence of 1 µM carbonylcyanide-p-trifluoromethoxyphenylhydrazon. State 3 and Respiratory Control Ratio (RCR) were calculated according to Chance and Williams [277].

6. Blood biochemistry

Plasma samples were collected and enzymatic determinations of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) performed using the Architect c System (Abbott Laboratories, USA).

7. Histology

Tissue samples analysis was performed on formalin-fixed paraffin-embedded tissue, using standard procedure: tissue samples were grossly inspected and sectioned, fixated in 4% formaldehyde, embedded in paraffin and cut in 4 µm sections. Examination by an experienced pathologist blinded to the experimental groups was performed on Haematoxylin and Eosin (H&E, Polysciences, Sakura Autostainer – Prisma 81D) stained slides observed in light microscope – Nikon Eclipse 50i, and

images obtained using a Nikon-Digital Sight DS-Fi1 camera. Sinusoidal dilation, cytoplasmic vacuolation, hepatocellular ballooning, hepatocellular cytoaggregation, nuclear pyknosis, steatosis, necrosis, polymorphonuclear inflammatory infiltrate, mitotic activity and billirubinostasis were graded as absent, mild, moderate and severe; in some cases the parameters were graded together as absent/mild versus moderate/severe.

B. Clinical study

Consecutive patients undergoing hepatectomy between January and September 2014 were included. Exclusion criteria were: refusal to participate, age under 18 years, laparoscopic hepatectomy, significant liver fibrosis (Metavir F3 or F4) and need for associated vascular resection. Institutional ethics committee approved the study and informed consent was obtained from each patient.

1. Study population and surgical procedures

Study population consisted of 30 patients, 16 male and 14 female, with a median age of 62 years and a median Model for End Stage Liver Disease (MELD) score of 8 (range 6–12). Indications were: colorectal cancer liver metastases in 16 cases (53.3%); non-colorectal cancer liver metastases in 7 (23.3%); cholangiocarcinoma in 3 (10%); and other benign indications in 4 (13.3%).

Hepatectomies were performed through bilateral subcostal incisions with routine used of intraoperative ultrasonography. Parenchymal transection was performed with ultrasonic dissection with CUSA™ Ultrasonic Surgical Aspirator (Integra, Plainsboro, N. USA) or Kelly-clamp crush technique, as previously described [115,273]. Twenty two (73%) patients underwent minor hepatectomies (up to two Couinaud segments) and eight (27%) major hepatectomies (three or more Couinaud segments). Hepatic pedicle clamping (HPC) was used (only when deemed necessary) in 21 (70%) patients, with a mean time of 39 ±25 minutes (range 10–99); an intermittent strategy of 15 minutes clamping with 5 minutes reperfusion was used. Sixteen (53%) patients underwent 20 or

more minutes of HPC. Liver parenchyma was normal in all but four patients with mild steatosis and in three with mild sinusoidal dilation. Clinical and operative data are detailed in Table 2.1.

2. Collection of biopsies

Study protocol consisted of taking 2 x 2 cm wedge biopsies in the non-resected liver at two time points: biopsy A, collected at the beginning of the procedure; and biopsy B, collected at the end of the procedure, immediately after the last period of clamping (if HPC was performed). Samples were divided. One fragment was placed in 4° C preservation solution (sucrose 250 mM, EGTA 0,5 mM, HEPES 10 mM, pH 7,4, BSA 1%) and immediately transported to the laboratory for mitochondrial fraction isolation and function tests: membrane potential and respiration. The other part of the biopsy was frozen at –80º C for assessment of ATP content.

3. Mitochondrial isolation, measurement of mitochondrial membrane potential and oxygen consumption

Mitochondrial isolation, membrane potential measurement and oxygen consumption were performed as described previously for the experimental study. Variables obtained were: initial membrane potential (mV); depolarization (mV); lag phase (seconds); and repolarization (mV). Readings were recorded in triplicate. Values for baseline sample (biopsy A) and end of surgery sample (biopsy B) were obtained and the difference was calculated as the value in sample B minus the value in sample A. Initial and final values of RCR were obtained and the difference calculated, as above.

4. Measurement of Adenosine Triphosphate (ATP) content

Liver ATP was extracted using an alkaline extraction procedure, as described by [278]. Tissue ATP levels were measured with the Luciferase / Luciferine assay (Sigma Chemical Company, St. Louis, MO) with a PerkinElmer VICTOR 3 plate-reader fluorometer (PerkinElmer, Waltham, MA), according with the manufacturer´s

instructions. Initial and final values of ATP were obtained and the difference calculated, as above.

Table 2.1 - Clinical and operative data of the study population submitted to liver resection (N= 30 patients undergoing hepatectomy for diverse indications)

	N	%
Male / Female	16 / 14	
Age (median, range)	62 years (42 – 80)	
Body Mass Index (mean ± standard deviation, range)	27.0 ± 4.9 $Kg.m^{-2}$ (20.0 – 38.0)	
Diabetes mellitus		
Yes	8	26.7
No	22	73.3
American Society of Anesthesiology classification		
I	8	26.7
II	16	53.3
III	6	20
Preoperative chemotherapy		
Yes	16	53.3
No	14	46.7
Indications		
Colorectal cancer liver metastases	16	53.3
Non-colorectal cancer liver metastases	7	23.3
Perihilar cholangiocarcinoma	3	10
Hepatocellular adenoma	1	3.3
Hydatid cyst	3	10
Surgical procedures		
Major hepatectomy	8	27%
• Right hepatectomy	4	13.3
• Extended right hepatectomy	1	3.3
• Left hepatectomy	2	6.7
• Central hepatectomy	1	3.3
Minor hepatectomy	22	73%
• Bissegmentectomy	4	13.3
• Segmentectomy	5	16.7
• Subsegmentectomy	7	23.3
• Multiple, atypical resections	6	20
Intraoperative transfusion		
Yes	6	20
No	24	80

5. Postoperative liver function and clinical course

Arterial lactate was measured every six hours in the first 24 hours. Standard biochemical determinations of International Normalized Ratio (INR), total bilirubin, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were performed on postoperative days 0, 1, 3, 5 and 7. Arterial lactate clearance was calculated according to Wu et al [279].

Postoperative morbidity was defined and graded according to Dindo et al up to the 90th postoperative day [280]. Posthepatectomy liver failure (PHLF), was defined and graded by International Study Group of Liver Surgery (ISGLS) consensus definition, as an increased value of both bilirubin and INR on or after the 5th postoperative day [162]. Bile leakage and biloma were defined by the ISGLS consensus [281]. Liver-specific morbidity was defined as any complication directly related to liver resection, namely PHLF, ascites, bleeding, bile leakage, biloma or intra-abdominal abscess.

C. Statistical analysis

All continuous variables were presented as mean ± standard error of the mean unless otherwise specified. Normality of distribution of continuous variables was assessed with Kolmogorov-Smirnov and Shapiro-Wilk tests, when indicated. Categorical variables were compared with the Chi-square test. Continuous variables with normal distribution were compared with Student t test and with non-normal distribution with the Mann-Whitney U test; correlations were assessed with Pearson correlation. Receiver operating characteristic (ROC) curve analysis was used to assess the predictive value of mitochondrial function for morbidity. Binary logistic regression was conducted to examine the individual effect of each parameter on postoperative morbidity. Statistical analysis was performed using SPSS™ (version 21.0, SPSS inc., Chicago, IL.). Significance was considered when $p < 0.05$.

III. Results

A. Experimental study

1. Effect of Hepatectomy with Hepatic Pedicle Clamping on Mitochondrial Oxidative Phosphorylation and Respiration

Animals in group 4 presented significantly increased lag phase at 12h posthepatectomy (122.4 ±19.6 seconds), indicating a longer time to repolarize the electrochemical gradient across the mitochondrial inner membrane, when compared with animals in other groups (92.2 ±4.1 seconds) (p=0.03) (Figure 2.1.A). However, no significant differences were observed in other bioenergetics parameters, including membrane potential, depolarization, repolarization or RCR.

2. Effect of Hepatectomy with Hepatic Pedicle Clamping on markers of hepatocellular death

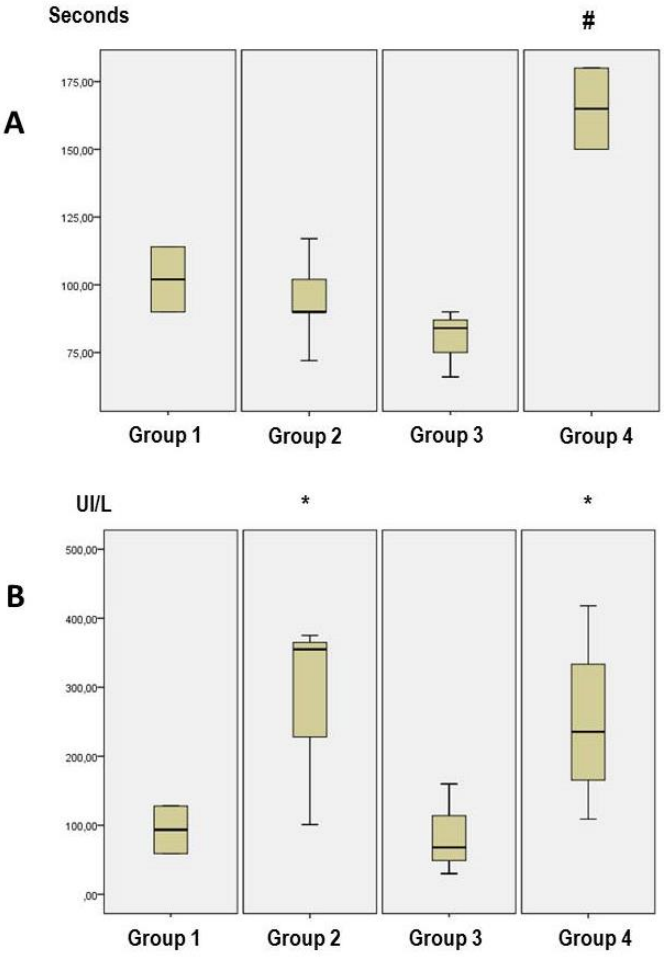
Both groups 2 and 4 presented a statistically significant increase in serum ALT (277 ±88.2 and 249 ±63.8 UI/L, respectively), when compared with groups 1 and 3 (93 ±34.5 and 86 ±38.6 UI/L, respectively) (p=0.03) (Figure 2.1.B). No statistically significant differences were observed in serum levels of AST or LDH.

3. Effect of Hepatectomy with Hepatic Pedicle Clamping on liver histology

As expected there was a significant increase in mitotic count at 24h and 48h in animals undergoing hepatectomy (groups 2 and 4) (median 1; IQR 1-4) versus animals not undergoing hepatectomy (groups 1 and 3) (median 0; IQR 0-0) (p=0.012). However, there was no statistically significant difference in remnant liver weight in goups 2 and 4. Macro and microvesicular steatosis at 48 hours was more prevalent in animals in group 4 than in the other groups (p=0.018) (Figure 2.2). No statistically significant difference in necrosis, ballooning, pyknosis, inflammatory cell infiltrate or sinusoidal dilation was observed between groups.

Furthermore, in groups 2 and 4, animals developing moderate or severe steatosis displayed reduced membrane potential (204.9 ±0.7) and RCR (3.3 ±0.3) versus animals developing no or mild steatosis (216.7 ±1.7 and 5.2 ±0.5, respectively) ($p<0.05$).

Figure 2.1 - Markers of mitochondrial function and hepatocellular necrosis in the experimental study: N= 35 Wistar rats undergoing sham operation (group1); intermittent hepatic pedicle clamping (HPC) (goup 2); 70% hepatectomy (group 3); or 70% hepatectomy with HPC (group 4)

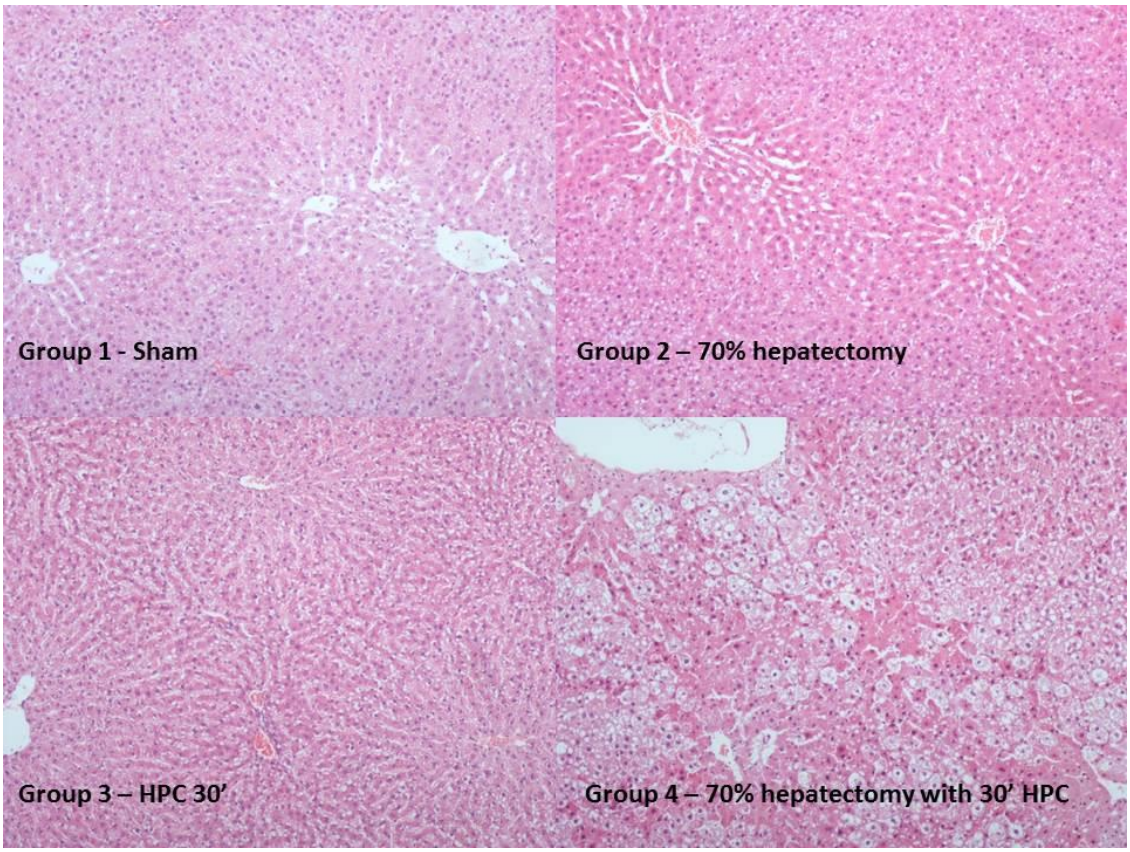


A. Mitochondrial function in different experimental groups: Lag phase was significantly longer (122.4 ±19.6 seconds) in animals in group 4 versus animals in other groups (# p=0.03 versus other groups)

B. Serum markers of hepatocellular necrosis: Alanine aminotransferase (ALT) was significantly increased in animais undergoing hepatectomy, irrespective of undergoing HPC (group 4) or not (group 2) (* *p*=0.03 versus groups 1 and 3)

Figure 2.2 - Histological analysis in the experimental study (N= 35 Wistar rats)

Representative findings on Haemotoxylin and Eosin staining of liver parenchyma 48 hours after surgery. Animals undergoing hepatectomy with hepatic pedicle clamping (HPC) (group 4) presented with increased micro- and macrovesicular steatosis versus other groups.



B. Clinical study

1. Clinical outcome

Major morbidity, defined as Dindo grades III-V, was present in eight (27%) patients. Five patients experienced PHLF (ISGLS grade A in four cases and grade C in one). Liver-specific complications were present in 11 (37%) patients: PHLF in five cases (17%); biloma in four cases (13%) (Dindo grade IIIA); bile leak in one case (3%) (grade II); hemorrhage in one case (3%) (grade II). Postoperative mortality occurred in one case (3%) due to grade C PHLF after extended right hepatectomy for cholangiocarcinoma. Median length of stay was 6 days (range 5 – 24).

2. Baseline mitochondrial function

We found a significant linear correlation between liver ATP levels with membrane depolarization (r= -0.693; p=0.039). Furthermore, patients with diabetes mellitus had a statistically significant lower baseline mitochondrial repolarization (178 ±5.4 mV; p=0.035) and lower Respiratory Control Ratio (1.86 ±0.3; p=0.035). Likewise, patients with obesity (body mass index > 30 $kg.m^{-2}$) had lower baseline Respiratory Control Ratio (1.85 ±0.2; p=0.034).

No statistically significant differences were observed in baseline mitochondrial function in patients with increasing age, gender, preoperative chemotherapy, steatosis or sinusoidal dilation.

3. Hepatic Pedicle Clamping and mitochondrial function

Longer HPC time significantly correlated with worsening mitochondrial oxidative phosphorylation, namely intraoperative decrease in mitochondrial membrane depolarization (r= -0.591; p=0.011) and intraoperative increase in lag phase (r= 0.568; p=0.006) (Figures 2.3 A, B and C). Patients with HPC time over 20 minutes suffered a significant intraoperative drop in depolarization (-3.06 ±1.7 mV) versus patients with less than 20 minutes of HPC, who experienced a mean increase (4.9 ±2.9 mV) (p=0.024).

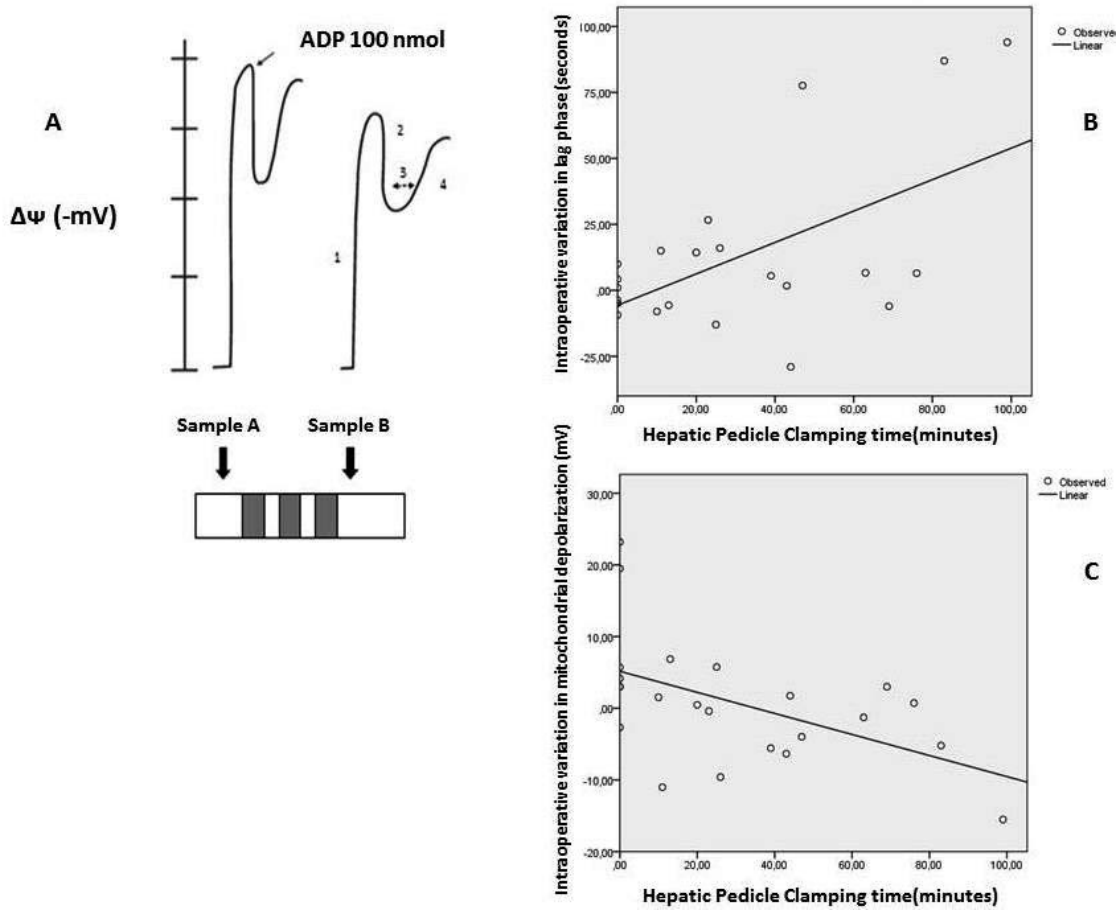
4. Mitochondrial function and hepatocellular necrosis and function

Mitochondrial bioenergetics presented several significant correlations with postoperative markers of hepatocellular necrosis (aminotransferases on day 1) and function (arterial lactate on the day of surgery, and INR and total bilirubin on day 5) (Table 2.2).

The stronger correlations observed were end of surgery depolarization with peak postoperative ALT (r= -0.568; p= 0.005) and AST (r= -0.574; p=0.004); and intraoperative change of lag phase with 5^{th} day INR (r= 0.550; p=0.008) and bilirubin (r= 0.679; p =0.001).

Figure 2.3 - Hepatic pedicle clamping and mitochondrial function in the clinical study (N= 30 patients undergoing hepatectomy for diverse indications)

A – Representative graph of mitochondrial functional parameters measured in the tetraphenylphosphonium (TPP^+) electrode in a patient with worsening of mitochondrial function from sample A (beginning of hepatectomy) to sample B (end of hepatectomy, after three 15 minutes periods of hepatic pedicle clamping): 1 – decrease in initial potential; 2 – decrease in depolarization; 3- longer lag phase; and 4 – decrease in repolarization
B - Correlation between longer hepatic pedicle clamping time and increase in lag phase in the study population (Pearson r = 0.568 p=0.006)
C – Correlation between longer hepatic pedicle clamping time and decrease in mitochondrial depolarization in the study population (Pearson r = -0.519 p=0.011)



The correlations between end of surgery depolarization and first day ALT and AST were stronger in patients with over 20 minutes of HPC (r= -824; p= 0.001 and r= -788; p=0.002, respectively).

In patients submitted to major hepatectomy RCR at the end of surgery significantly correlated with arterial lactate clearance (r= 0.756; p=0.049) and variation in mitochondrial potential also correlated negatively and strongly with first day arterial lactate (r= -0.892; p= 0.042).

Table 2.2 - Mitochondrial function and postoperative liver function in the clinical study (N= 30 patients undergoing hepatectomy for diverse indications)

Correlations between mitochondrial bioenergetics parameters and markers of hepatocellular function and necrosis (arterial lactate 6 hours after end of surgery, peak postoperative aminotransferases on postoperative day 1, International Normalized Ratio on postoperative day 5 and total bilirubin on day 5) in the study population (* $p < 0.05$; ** $p < 0.005$)

	Arterial lactate 6 h	Day 1 ALT	Day 1 AST	Day 5 Bilirubin	Day 5 INR
End of surgery membrane potential	$r = 0.068$	$r = -0.326$	$r = -0.225$	$r = -0.015$	$r = -0.219$
End of surgery lag phase	$r = 0.452$*	$r = 0.452$*	$r = 0.415$*	$r = 0.444$*	$r = 0.627$**
End of surgery depolarization	$r = -0.282$	$r = -0.568$*	$r = -0.574$**	$r = -0.179$	$r = -0.224$
End of surgery repolarization	$r = -0.095$	$r = -0.096$	$r = 0.003$	$r = 0.055$	$r = -0.368$
Change in membrane potential	$r = -0.357$	$r = -0.453$*	$r = -0.445$*	$r = -0.416$	$r = -0.343$
Change in lag phase	$r = 0.508$*	$r = 0.484$*	$r = 0.460$*	$r = 0.550$*	$r = 0.679$**
Change in depolarization	$r = -0.426$*	$r = -0.571$**	$r = -0.553$*	$r = -0.279$	$r = -0.257$
Change in repolarization	$r = -0.287$	$r = -0.112$	$r = -0.096$	$r = -0.332$	$r = -0.494$*

5. Mitochondrial function and postoperative morbidity

Patients developing liver-specific morbidity and PHLF presented with worse end of surgery mitochondrial bioenergetics (Table 2.3).

Multivariate analysis demonstrated that intraoperative deterioration in mitochondrial membrane potential (HR=13.7; p=0.043) and preoperative chemotherapy (HR=13.7; p=0.043) were independent risk factors for liver-specific morbidity (Table 2.4).

Table 2.3 - Mitochondrial bioenergetics and postoperative morbidity in the clinical study (N= 30 patients undergoing hepatectomy for diverse indications)

Intraoperative measurements of mitochondrial bioenergetics (sample A, sample B and intraoperative change) in the study population and in patients developing postoperative complications (liver-specific morbidity and posthepatectomy liver failure). Mann-Whitney two-tailed: a) p = 0.037; b) p = 0.022; c) p = 0.032; d) p = 0.004; e) p = 0.018; f) p = 0.011; g) p = 0.037

	Study population (N = 30)			**Liver-specific morbidity (n = 11)**			**Posthepatectomy Liver Failure (n = 5)**		
Parameter	**Biopsy A**	**Biopsy B**	**Variation**	**Biopsy A**	**Biopsy B**	**Variation**	**Biopsy A**	**Biopsy B**	**Variation**
Membrane potential (mV)	193.5 ±2.5	**191.9 ±2.7** [a)]	**- 0.9 ±3.1** [b)]	196.4 ±3.2	185.1 ±4.7	- 7.7 ±5.5	200.1 ±3.7	**178.8 ±6.9** [a)]	**- 17.1 ±7.8** [b)]
Lag phase (sec)	41.9 ±3.1	**54.7 ±5.9** [c), d)]	**+ 13.0 ±6.8** [e), f)]	39.3 ±3.1	**73.3 ±12.4** [c)]	**+ 33.9 ±13.6** [e)]	40.5 ±4.8	**95.3 ±15.1** [d)]	**+54.8 ±19.4** [f)]
Depolarization (mV)	21.6 ±1.1	22.3 ±1.0	**+ 0.7 ±1.8** [g)]	24.3 ±1.9	20.4 ±1.1	**- 3.9 ±2.3** [g)]	24.1 ±2.4	19.2 ±1.6	- 4.9 ±3.4
Repolarization (mV)	187.1 ±2.6	185 ±2.5	- 1.3 ±3.2	191.7 ±1.9	182.4 ±3.8	- 9.2 ±4.9	192.6 ±3.1	177.8 ±3.8	- 15.3 ±5.4
Respiratory Control Ratio	2.4 ±0.2	2.7 ±0.2	+ 0.3 ±0.2	2.7 ±0.2	2.7 ±0.4	+ 0.2 ±0.3	2.8 ±0.4	2.8 ±0.7	+ 0.1 ±0.6

Table 2.4 - Univariate and multivariate analysis of factors associated with liver-specific morbidity in the clinical study (N= 30 patients undergoing hepatectomy for diverse indications)

	Univariate analysis			**Multivariate analysis**		
	OR	**CI 95%**	**p**	**OR**	**CI 95%**	**p**
Preoperative chemotherapy	3.67	0.7 – 18.3	0.107	**13.7**	**1.1 – 173.7**	**0.043**
Major hepatectomy	**7.1**	**1.1 – 46.7**	**0.043**	_	_	*n.s.*
HPC > 20 minutes	**7.7**	**1.28 – 46.4**	**0.021**	_	_	*n.s.*
Decrease in mitochondrial membrane potential	6.3	0.9 – 42.7	0.06	**13.7**	**1.1 – 173.7**	**0.043**

Elevated lag phase at the end of surgery, above 53.9 seconds, was found to be highly predictive of PHLF (area under the curve: 0.933; p=0.008; sensitivity 100%; specificity 83.3%). However, there was no statistically significant association of mitochondrial dysfunction with major morbidity or mortality.

IV. Discussion

Posthepatectomy liver failure is a severe complication of liver surgery, associated with increased mortality, risk of sepsis and decreased long term survival [13,14,282]. Liver regeneration is a highly energy dependent process and bioenergetic status of the main parenchymal cell of the liver, the hepatocyte, is of paramount importance in this process. However, the exact role of bioenergetics in clinical posthepatectomy liver dysfunction is largely unknown.

Experimental evidence confirms the key role of mitochondrial homeostasis in liver regeneration. Improved energy status and decreased mitochondrial permeability transition can improve liver regeneration, maintain hepatocellular function and decrease mortality in animal models of major hepatectomy [20,52]. However, clinical evidence for bioenergetics dysfunction in liver surgery is scarce. One study failed to show significant correlations of mitochondrial respiration with clinical parameters, possibly because only one intraoperative biopsy was made [65]. Furthermore, the effect of hepatic pedicle clamping on mitochondrial function in the setting of liver resection has not been fully clarified, both clinically and experimentally. This led to the design of two distinct and original studies, one experimental and one clinical, to elucidate these issues.

In the clinical study we sought to investigate the change in liver mitochondrial function by performing two intraoperative biopsies in the non-resected parenchyma, one at the beginning of the procedure and one at the end. Rather than only measuring the absolute value, we also measured the change in mitochondrial membrane potential, oxygen consumption and ATP content between the two samples, thus reflecting the intraoperative change in the liver energy state. We then sought to correlate these data with preoperative liver function, HPC time, markers of hepatocellular necrosis and function, and postoperative morbidity.

Unsurprisingly, there was a significant correlation between mitochondrial membrane depolarization and ATP levels. There is a direct and biological rationale for this finding, since ATP synthesis depends on proper function of the electron transport chain, as well as adequate adenosine diphosphate transport into the mitochondrial matrix and an efficient ATP-synthase.

We were not able to demonstrate any correlation of mitochondrial metabolism with preoperative liver function. This is easily explained, not only because cirrhotic patients were excluded from the study, but also because liver parenchyma was normal all but in seven patients, who presented mild histologic changes; this was confirmed by the low median MELD score of the study population. Regarding the effect of other clinical variables on mitochondrial function, we also did not confirm any change with advanced age or preoperative chemotherapy. Nonetheless, our results confirmed that patients with diabetes and obesity had worse mitochondrial function and this finding is in keeping with other studies [56,272] (and reviewed in **Chapter I -** "*Bioenergetics and Liver Regeneration: Review of the Role of Energy Status in the Pathophysiology of Posthepatectomy Liver Failure*").

Regarding the association of HPC with mitochondrial dysfunction, answers were sought in both the clinical and the experimental study.

The experimental study was designed to explore the effect of a well-tolerated HPC strategy, namely 30 minutes intermittent clamping strategy. Although this duration of HPC, if used continuously, is associated with increased hepatocellular injury in animal models of 70% hepatectomy [220,283], it is well tolerated when used intermittently [284]. Furthermore, intermittent clamping is the most widely used strategy in the clinical setting [68,274,285]. We confirmed little evidence for increase in hepatocyte death, as was demonstrated by similar ALT levels in both hepatectomy groups, irrespective of HPC. Group 4 also did not present an enhanced necrosis score on histology when compared with animals undergoing hepatectomy without HPC. Nonetheless, animals undergoing hepatectomy with HPC presented longer lag phase at 12 hours posthepatectomy, reflecting an impaired phosphorylation of ADP. Group 4 also demonstrated an increased steatosis score at 48 hours, versus the other experimental groups. Another interesting finding was that both groups of animals

undergoing hepatectomy presented an association of intracellular accumulation of lipids with decreased mitochondrial respiration and membrane potential. In fact, steatosis is known to occur during early liver regeneration and improvement in energy status can potentially decrease intra-hepatocyte lipid accumulation [22,286]. These findings could be easily explained, as hepatocytes become preferential users of free fatty acids released from adipose tissue after surgical aggression. So, a disturbance in the mitochondrial electron transport chain and subsequent decreased oxygen consumption would lead to an increase in the reduced form of nicotinamide adenine dinucleotide (NADH) and increased NADH/NAD+ ratio, thus halting the β-oxidation of fatty acids, causing their accumulation in the hepatocytes' cytoplasm.

As such, we demonstrated that mitochondrial dysfunction, in particular longer lag phase, is a subtle yet measurable biologic change of the liver parenchyma after hepatectomy with inflow occlusion in the experimental model. We postulate that longer HPC time is associated with increased mitochondrial dysfunction, and this was confirmed in the clinical study. In the cohort of patients we observed a moderately strong and statistically significant correlation of duration of HPC with two relevant bioenergetics variables. Furthermore, mitochondrial membrane depolarization was significantly worsened in patients with over 20 minutes of HPC, whereas it was improved in patients with under 20 minutes, possibly reflecting a mechanism of ischemic preconditioning [108]. This confirms the previous notion that a strategy of intermittent HPC in an on-need basis is usually considered safest [273,274]. Nevertheless, HPC time over 20 minutes has previously been associated with an increased risk of liver failure and mortality [287].

In keeping with the second interrogation of this study, our clinical results clearly demonstrate a significant relationship between mitochondrial bioenergetics with postoperative markers of hepatocellular necrosis. There was a moderate correlation between deterioration of mitochondrial depolarization and peak postoperative aminotransferases. This correlation was even stronger in patients undergoing HPC over 20 minutes, possibly reflecting increased hepatocyte necrosis caused by bioenergetic failure. Although peak postoperative aminotransferases do not necessarily reflect outcome [288], they are a useful surrogate of remnant liver ischemia-reperfusion injury.

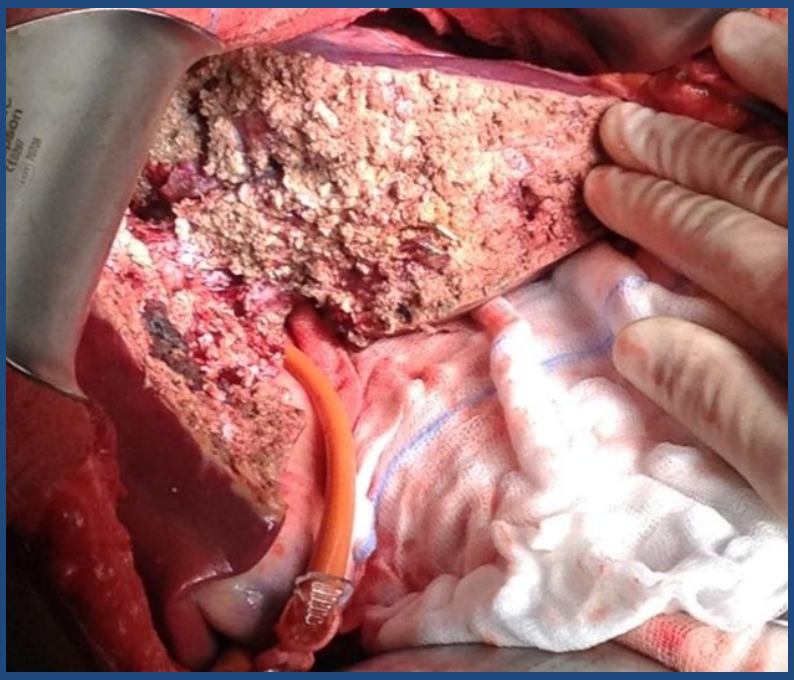
An additional finding was the important correlation of mitochondrial dysfunction with two key postoperative markers of hepatocellular function, namely fifth day INR and bilirubin [165,289]. Furthermore, patients undergoing major hepatectomy also experienced a very strong correlation between mitochondrial dysfunction and arterial lactate on postoperative day 1, a sensitive marker of liver function [171,279]; and between decreased oxygen consumption and depressed lactate clearance, possibly reflecting lower hepatic lactate metabolism rather than increased peripheral production [172].

One of the main results of our study was a strong association of mitochondrial dysfunction with PHLF. Patients with PHLF presented lower end of surgery membrane potential and increased lag phase, likely reflecting the development of bioenergetics failure. In fact, end of surgery lag phase was a strong predictor of PHLF, with high specificity and sensitivity. And finally, intraoperative decrease in mitochondrial membrane potential was an independent risk factor for liver-specific morbidity.

This clinical study is, to the best of our knowledge, the first to demonstrate a clear relationship between mitochondrial function and clinical outcome after hepatectomy. The importance of these findings is twofold. First, markers of mitochondrial dysfunction can be used for earlier diagnosis of PHLF. And finally, research into the mechanisms of bioenergetics dysfunction in liver surgery could open the way to novel pharmacological therapies. If translated into clinical practice, improvement in hepatocyte bioenergetics could decrease susceptibility to ischemia-reperfusion injury, accelerate liver regeneration and ultimately decrease morbidity and mortality of liver resection. These concepts were explored in detail in **Chapter I** - "*Bioenergetics and Liver Regeneration: Review of the Role of Energy Status in the Pathophysiology of Posthepatectomy Liver Failure*".

Colour Plates

Plate I: Bloodless parenchymal transection during left hepatectomy for malignancy



Profound knowledge of liver anatomy, technological advances in surgical instruments and dedicated anesthetic and perioperative care have decreased the risk of intraoperative bleeding during parenchymal transection. Nowadays clinical success of hepatectomy depends mostly on the capacity of the remnant liver to regenerate.

Thus, constraints to resection are usually not imposed by the hepatic tumour load but by the quality and volume of the future liver remnant. When the liver's capacity for regeneration is surpassed, Posthepatectomy Liver Failure ensues.

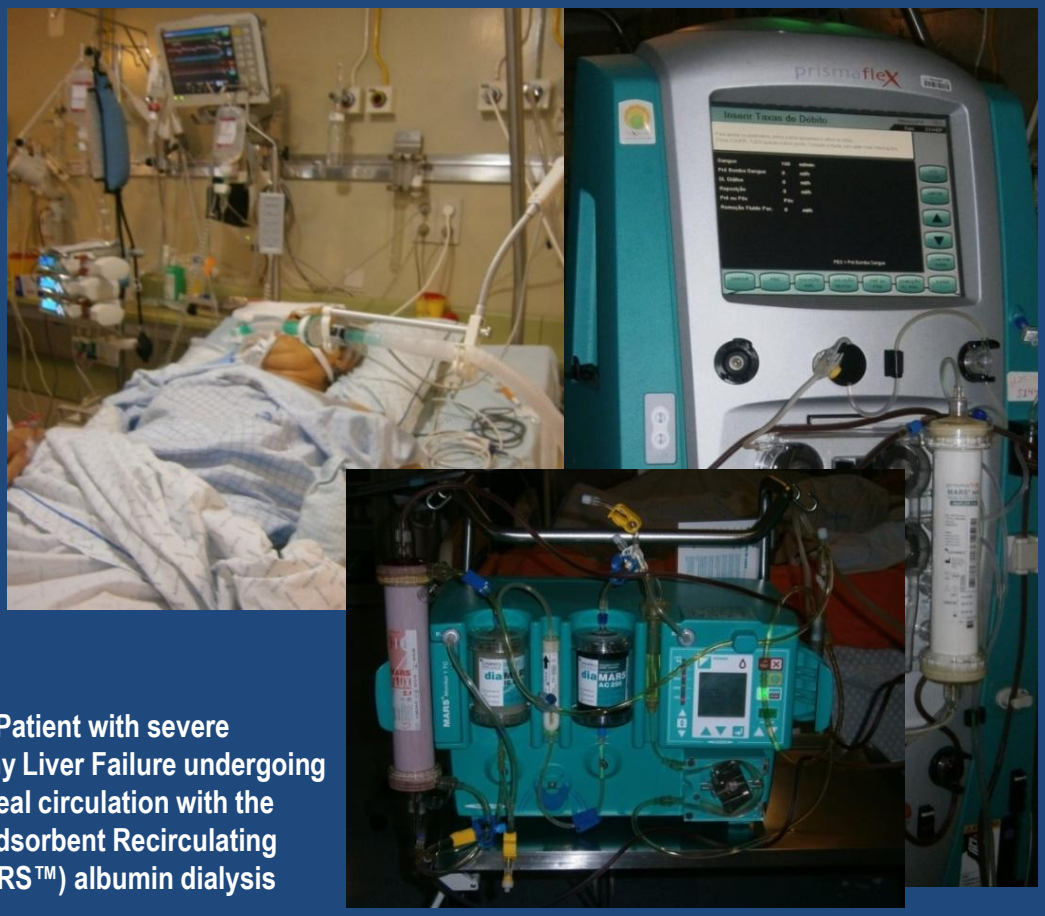


Plate II: Patient with severe Posthepatectomy Liver Failure undergoing extracorporeal circulation with the Molecular Adsorbent Recirculating System (MARS™) albumin dialysis

Posthepatectomy liver failure (PHLF) is the most dreaded complication of liver resection. Treatment is disappointing, even in spite of technological advances which include extracorporeal albumin dialysis systems. Clinically PHLF is characterized by ascites, jaundice and renal impairment. Sepsis is a common complication and sometimes a preterminal event, possibly functioning as a second-hit to an already compromised liver parenchyma.

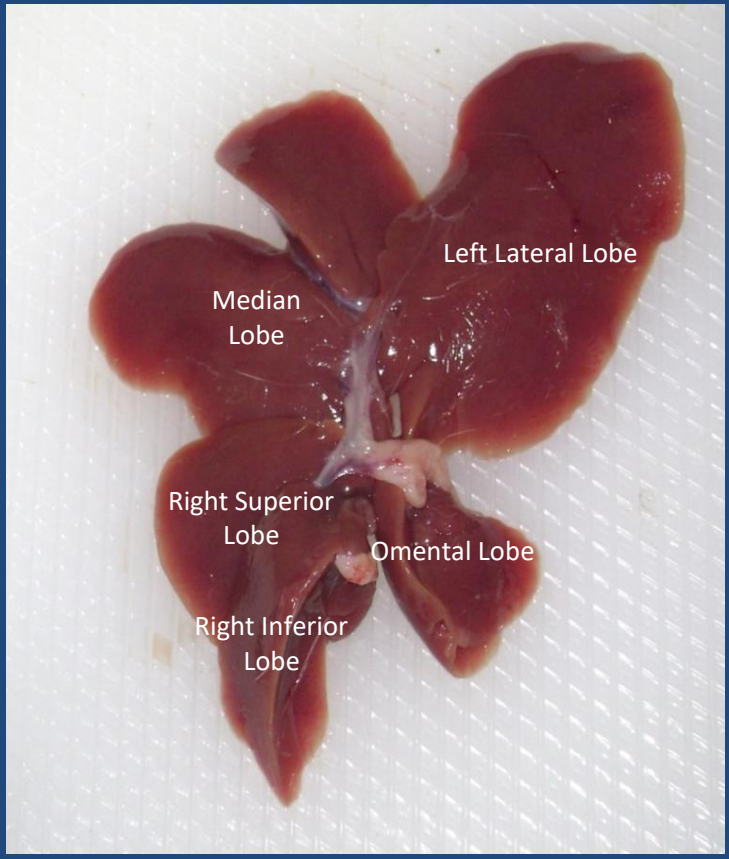
While mitochondrial dysfunction has been demonstrated to be involved in the pathogenesis of acute liver failure in other etiologies, this link has remained unproven in the setting of PHLF until recently.

The focus of both basic and clinical research has been on understanding the pathophysiology of PHLF in order to prevent its occurrence.

Plate III: Normal liver anatomy of the Wistar rat

The lobular structure and remarkable regenerative capacity of the rat liver provide an excellent model for the study of liver regeneration. The most used experimental procedure is the 70% hepatectomy model, which involves resection of the left lateral and median lobes. Liver regeneration starts almost immediately after resection and is usually complete by the seventh postoperative day.

Previous experimental works prove that liver energy status is a relevant factor in this biological process.



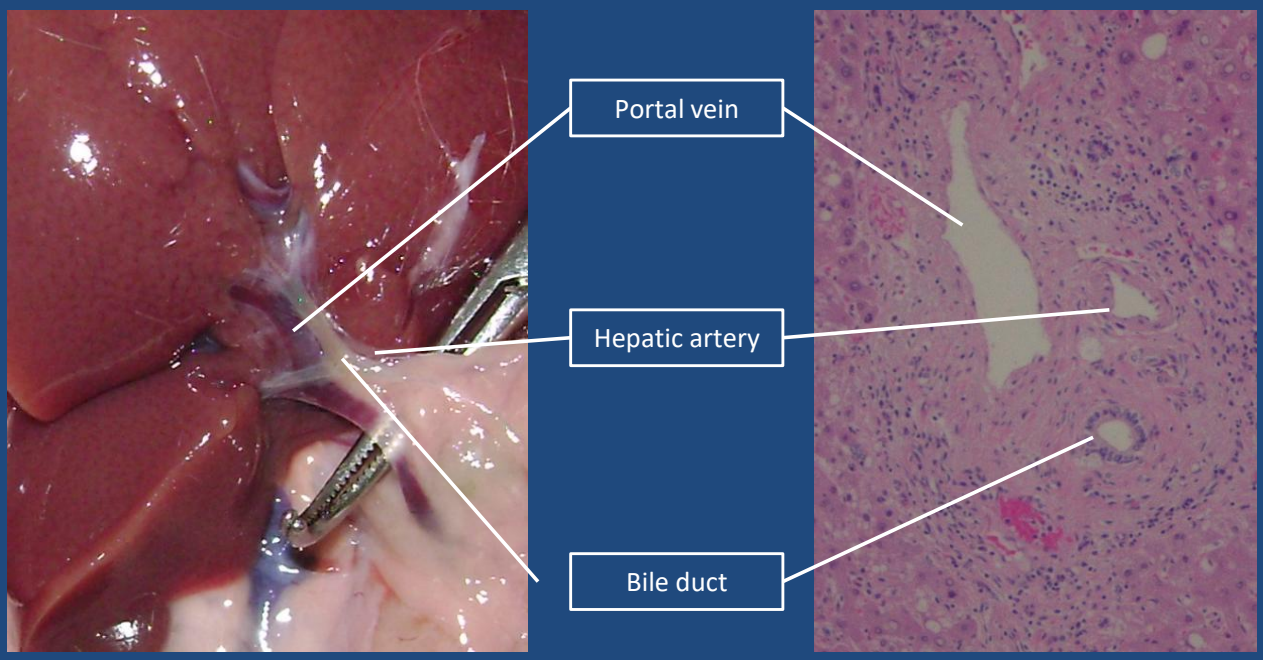


Plate IV: Anatomy of the liver hilum of the Wistar rat and the microscopic appearance of the portal triad – The vasculo-biliary tree, from the hilum to the acinus

The liver parenchyma is supported by portal and arterial inflow, biliary drainage and venous outflow. From the liver pedicle (left) to the most fundamental functional unit – the acinus – portal, arterial and biliary branches sequentially bifurcate in sectorial, segmental and subsegmental branches, supplying the entire liver mass. At the microscopic level this is exemplified by the portal triad (right, Hematoxylin-Eosin 100x), the lowest division of the elements of the hepatic pedicle, where the vasculo-biliary structures are enveloped in a connective tissue sheath that is contiguous with the Glisson's capsule.

The portal vein supplies the liver sinusoids with blood from the splanchnic organs, ensuring first contact of the liver with absorbed nutrients and xenobiotics, high concentration of entero-hormones and recirculating bile salts. The hepatic artery, on the other hand is responsible for the continuous delivery of oxygen, indispensable for the extremely high metabolic demands of the liver in its tireless regulation of glucidic, proteic and lipidic metabolism.

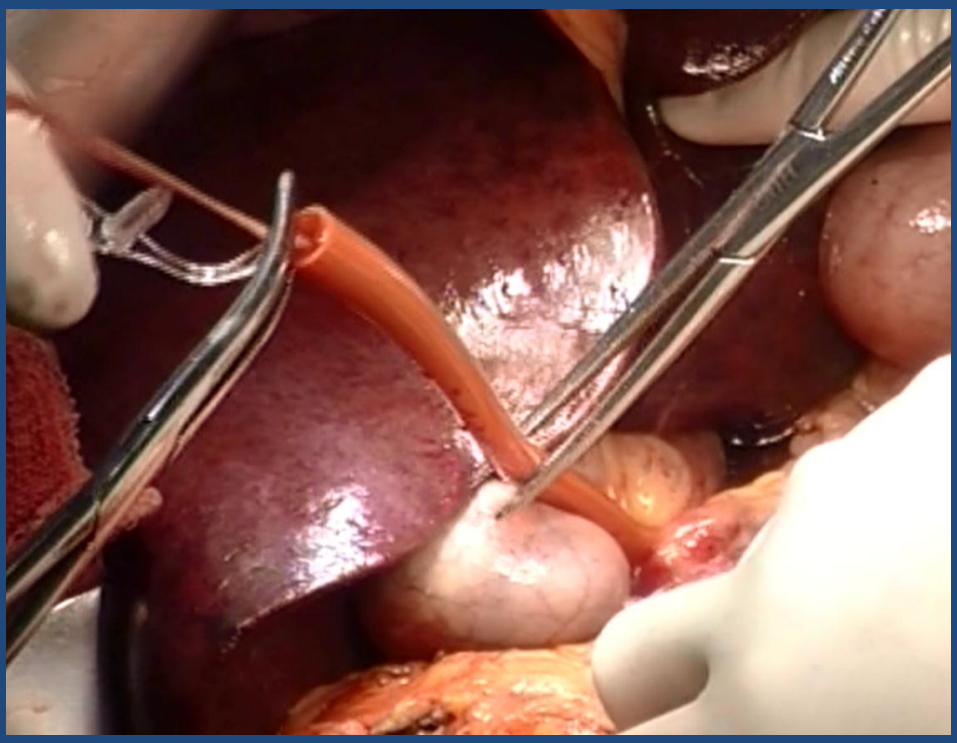


Plate V: Hepatic Pedicle Clamping in a patient with "Blue Liver"

Intraoperative appearance of Sinusoidal Obstruction Syndrome (SOS), or "Blue Liver", in a patient undergoing hepatectomy for colorectal cancer liver metastases after oxaliplatin-based chemotherapy. SOS is associated with increased risk of intraoperative bleeding and complications after hepatectomy.

In order to reduce bleeding in clinical liver resection or liver trauma, the hepatic pedicle can be temporarily clamped, a manoeuvre first described by Dr. John Hogarth Pringle, of Glasgow, in 1908. Hepatic Pedicle Clamping is an invaluable adjunct as it reduces intraoperative bleeding but it should be used judiciously in patients with chronic liver disease, including SOS, due to an increased susceptibility to ischemia-reperfusion injury and mitochondrial dysfunction.

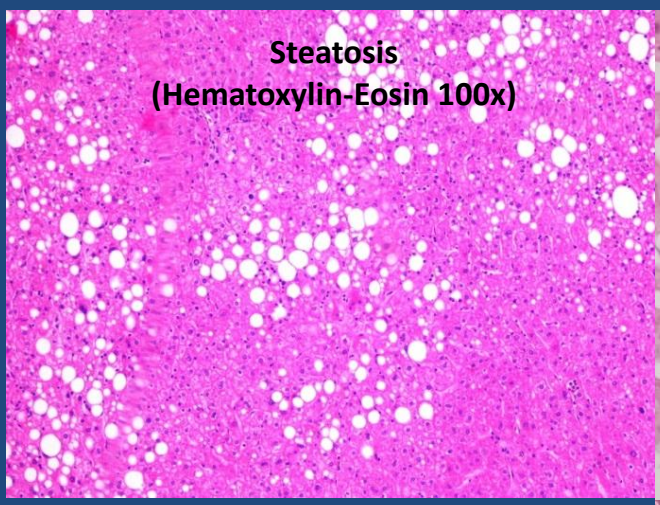


Plate VI: Histologic patters of Chemotherapy-associated liver injury (CALI)

Preoperative chemotherapy is commonly used in the management of patients with colorectal cancer liver metastases, in order to downsize lesions and improve survival. However, hepatotoxicity is a concern and parenchymal injury can compromise liver regeneration and increase morbidity and mortality of liver resection.

Three distinct patterns are recognized on liver histology: simple steatosis, steatohepatitis and sinusoidal obstruction syndrome. Disturbed cellular bioenergetics could prove to be a contributing factor in the pathophysiology of these conditions.

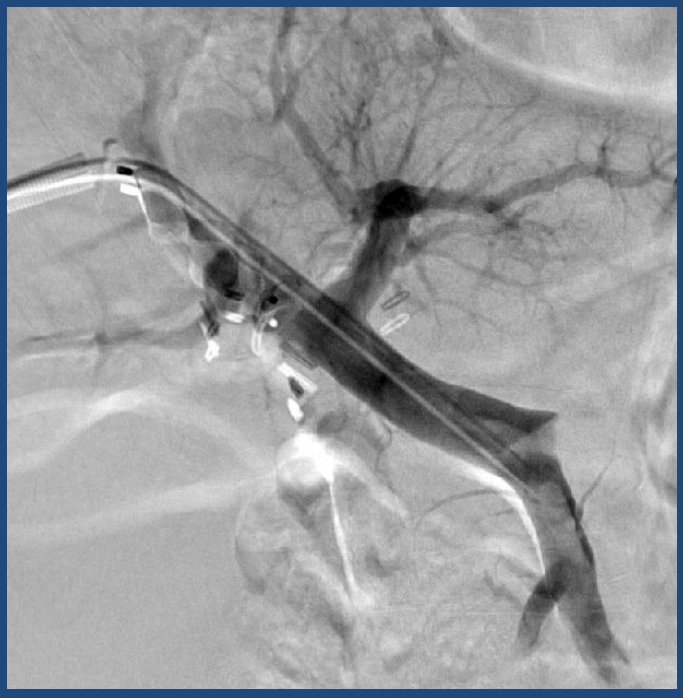


Plate VII: Percutaneous Right Portal Vein Embolization before Right Hepatectomy

To decrease the risk of Posthepatectomy Liver Failure after major or extended liver resection, Portal Vein Embolization (PVE) is occasionally required. This technique was first described in 1986 and is usually performed under fluoroscopic guidance, through direct liver puncture.

By shunting portal blood flow to the non-tumor bearing parenchyma, PVE causes atrophy and apoptosis in the embolized hemiliver; and cell proliferation and hypertrophy in the non-embolized future liver remnant. After some weeks major hepatectomy can be performed with decreased morbidity.

Experimental evidence suggests that important adaptations in mitochondrial function take place in the non-embolized parenchyma.

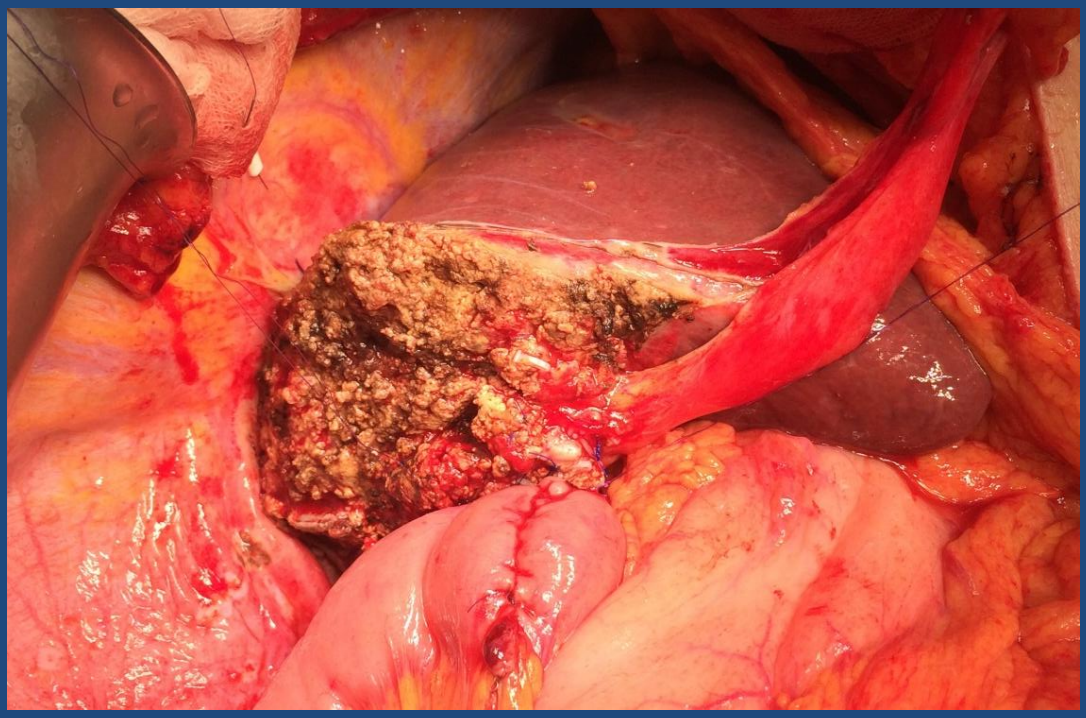


Plate VIII:

Liver remnant consisting of segments 2 and 3 after right extended hepatectomy with bile duct resection for Bismuth type IV hilar cholangiocarcinoma

Extended resections, i.e. resections of five or more Couinaud segments, are one of the most risky procedures in modern Hepatobiliary Surgery, on occasion resulting in Posthepatectomy Liver Failure.

Hilar cholangiocarcinoma is a particularly challenging condition, because patients usually present extremely jaundiced and biliary obstruction can severely hamper the liver remnant's capacity for regeneration. Both experimental and clinical evidence suggest that liver mitochondrial function is deteriorated in chronic biliary obstruction and can significantly recover after biliary drainage.

While meticulous surgical technique is mandatory, preoperative optimization of the future liver remnant is also a critical factor in the success of liver resection. This patient underwent the following consecutive preoperative percutaneous interventions: external biliary drainage; right and segment 4 portal vein embolization; and middle hepatic vein occlusion. With these procedures the future liver remnant volume increased from 222 cm^3 to 360 cm^3 making radical resection possible. She was discharged home on postoperative day 9 without any signs of liver dysfunction.

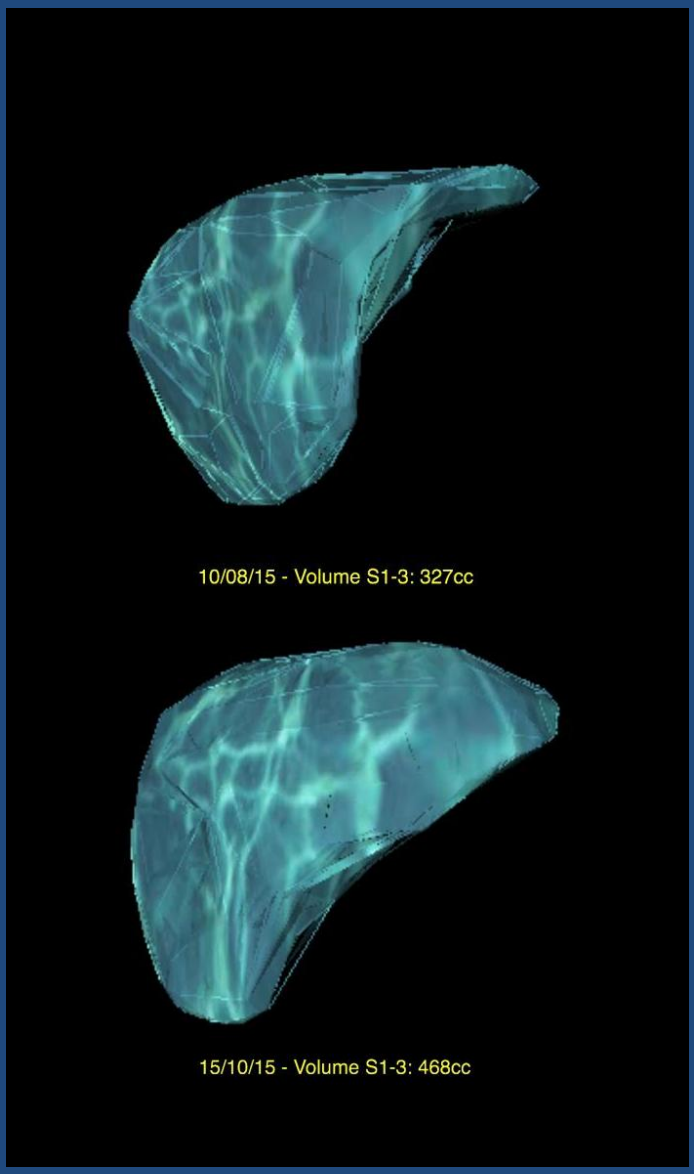
Plate IX: Volumetric analysis of the future liver remnant with the Osirix™ software before and after stage 1 of the ALPSS procedure

Volumetric analysis by cross sectional imaging is the gold standard for the correct assessment of the response to Portal Vein Embolization or two-stage procedures, like the Associating Liver Partition and Portal Ligation for Staged Hepatectomy (ALPPS).

Recent clinical evidence has shown that rather than the absolute increase in volume, the main factor in predicting regenerative capacity might be the kinetic pattern of liver growth.

In the case illustrated the future liver remnant sustained a volume increase of 43% in less than two weeks and the patient underwent a non-eventful recovery after the second stage of ALPPS.

Being a highly-energy dependent chain of events, liver regeneration is influenced by mitochondrial status. In fact, sufficient evidence exists for a definite link of mitochondrial oxidative phosphorylation in the enhanced inter-stages liver volume growth in ALPPS.



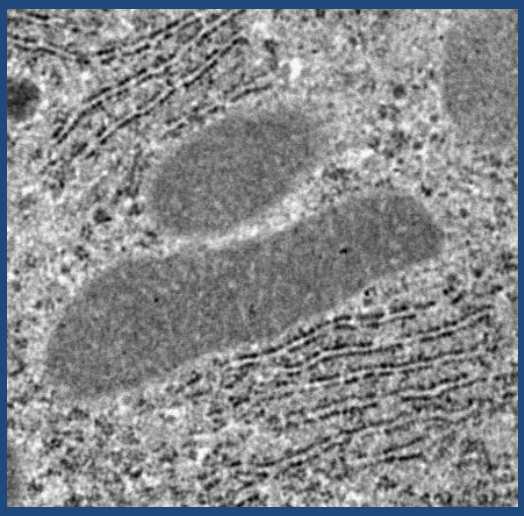


Plate X: Mitochondria – the engine behind the liver's amazing regenerative capacity

The liver's extremely high metabolic demands can only be maintained through the unremitting work of mitochondria. Most liver functions are endergonic, or energy-dependent, requiring substantial amounts of adenosine triphosphate, supplied predominantly by mitochondrial oxidative phosphorylation under aerobic conditions.

After hepatectomy the remaining hepatocytes must replace lost liver mass while still maintaining liver function. This can only happen with an increased energy supply by mitochondria.

Potentially, liver functional reserve before hepatectomy could be quantified through the measurement of metabolic capacity, instead of using single-function tests, like indocyanine green retention rate or hepatobiliary scintigraphy.

Furthermore, mitochondrial-based therapies could plausibly increase the liver's regenerative capacity and decrease the incidence of Posthepatectomy Liver Failure, surpassing the current limits of liver resection and providing hope for cure to patients nowadays deemed inoperable.

Chapter III - Chemotherapy Associated Liver Injury and Posthepatectomy Morbidity: Possible contribution of Mitochondrial Dysfunction to the Pathogenesis of Sinusoidal Obstruction Syndrome

Part of this chapter has been published as:

Oxaliplatin toxicity presenting as a liver nodule - case report.
Alexandrino H, Oliveira D, Cipriano MA, Ferreira L, Tralhão JG, Castro E Sousa F.
BMC Cancer. 2015 Apr 10;15:247. doi: 10.1186/s12885-015-1247-4.

Sinusoidal dilation increases the risk of complications in hepatectomy for CRCLM - Protective effect of bevacizumab and diabetes mellitus, serum gamma-glutamyltranspeptidase as predictive factor.
Martins J, Alexandrino H, Oliveira R, Cipriano MA, Falcão D, Ferreira L, Martins R, Serôdio M, Martins M, Tralhão JG, Prado e Castro L, Castro E Sousa F.
Eur J Surg Oncol. 2016 May;42(5):713-21. doi: 10.1016/j.ejso.2016.02.017.

Chemotherapy-Associated Liver Injury, Mitochondrial Dysfunction and Posthepatectomy Liver Failure – Exploration of a Possible Link in an Animal Model
Alexandrino H, Soeiro Teodoro J, Caetano Oliveira R, Cardoso J, Cipriano MA, Tralhão JG, Rolo A, Castro e Sousa F, Palmeira C
Journal of Translational Medicine (submitted)

I. Introduction

Yearly, over 447.000 new cases of colorectal cancer are diagnosed in Europe and around 50% of them will develop liver metastases [290]. Liver resection is potentially curative in patients with colorectal cancer liver metastases (CRLM) [90,291], but up to 85% of patients are considered unresectable due to tumor load [2]. In these cases hepatectomy can be performed after aggressive chemotherapy in a conversion strategy, downsizing the lesions and allowing for safe resection [2,90]. Moreover, neoadjuvant chemotherapy (NCT) in upfront operable patients can improve overall and disease-free survival after hepatectomy [130,292]. Modern NCT consists of 5-fluorouracil (5-FU) in association with irinotecan (FOLFIRI) or oxaliplatin (FOLFOX). Molecular-targeted agents, such as cetuximab and bevacizumab, have improved response rates and resectability [293].

However, NCT is known to cause chemotherapy-associated liver injury (CALI) and may increase morbidity and mortality after liver resection [116,133,294]. Irinotecan has been reported to cause steatohepatitis (SH) [116], while oxaliplatin-based chemotherapy has been associated with sinusoidal obstruction syndrome [136,138,295,296]. Sinusoidal obstruction syndrome (SOS) consists of severe sinusoidal dilation and congestion, sinusoidal endothelial cell necrosis and sloughing, erythrocyte extravasation in the sinusoids, peliosis and decreased sinusoidal flow [136,137]. SOS is associated with depressed preoperative liver function, increased intraoperative bleeding, decreased tolerance to hepatic pedicle clamping (HPC), worse posthepatectomy liver function and lower regenerative capacity after two-stage hepatectomy or portal vein embolization [138–143,297].

Focal peliosis in SOS can also cause another unexpected side effect, which is the development of liver nodules on cross-sectional imaging that can be mistaken for liver metastases. We have previously reported the case of a 59 year old male patient with T3N1bM0 sigmoid colon adenocarcinoma [298]. The patient underwent sigmoidectomy and adjuvant chemotherapy with capecitabine and oxaliplatin. One year after surgery, a

26 mm hypodense de novo liver nodule on segment 8 suggestive of liver metastasis was discovered on routine postoperative computed tomography (CT) (Figure 3.1 A and B). A presumptive diagnosis of CRLM was made and the patient underwent uneventful right hepatectomy. Pathological examination demonstrated no evidence of nodule, with a congestive liver. Histologically there was intense sinusoidal congestion and dilatation with areas of hemorrhage, trabecular atrophy, focal peliosis and perisinuosoidal fibrosis, diagnostic of SOS (Figures 3.1 C through F).

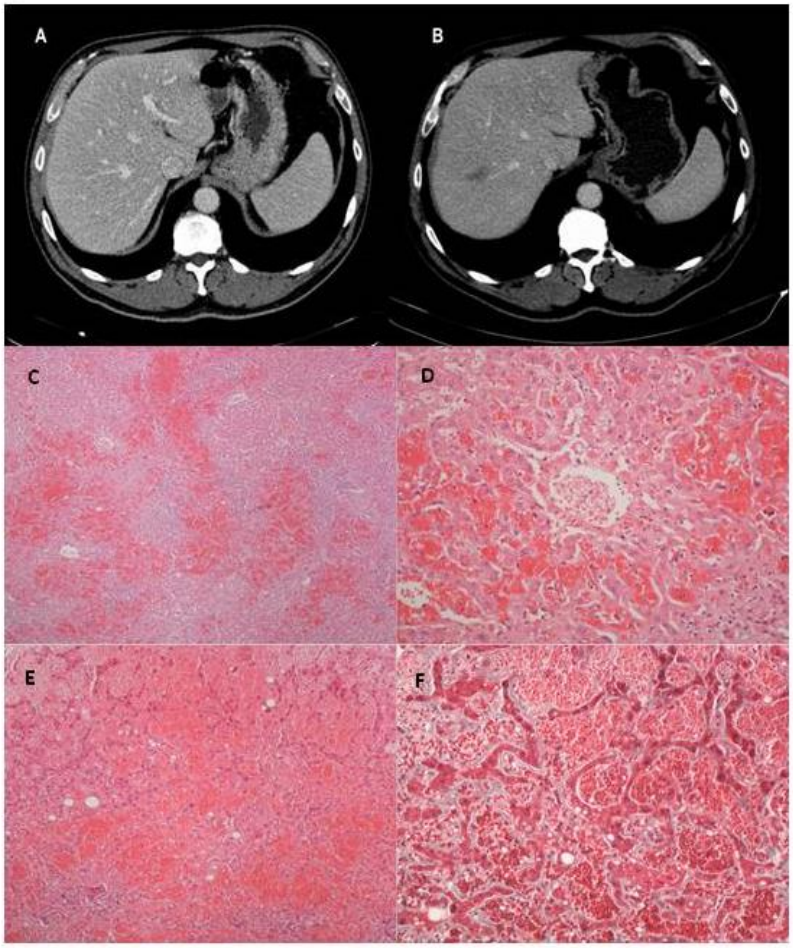


Figure 3.1 - A case of severe sinusoidal obstruction syndrome mimicking liver metastases: radiological and pathological presentation

A - Preoperative contrast enhanced abdominal computed tomography (CECT) showing no liver nodules
B - CECT after 12 cycles of oxaliplatin-based adjuvant chemotherapy showing a new hypodense liver nodule in segment 8, in close proximity to the right hepatic vein
C - Panlobular diffuse sinusoidal dilatation and congestion (Hematoxylin-Eosin H&E 40x)
D - Normal terminal hepatic veins (H&E 200x)
E - Extreme sinusoidal dilatation with peliosis (H&E 100x)
F - Atrophic hepatocelular plates and perisinusoidal fibrosis (Trichrome Masson 200x)

The finding of liver nodules mimicking liver metastases is an extremely rare event, and to our knowledge only two other publications have described this event previously to our paper [299,300]; but since then other cases have been reported [301,302]. These cases reinforce the need for an accurate imaging assessment of

patients undergoing NCT before hepatectomy. In this regard, gadoxetic acid enhanced and diffusion-weighted Magnetic Resonance is gaining increasing relevance, not only by detecting typical radiologic signs of SOS such as hypointense reticulations and increased spleen size, but also by staging preoperative liver reserve [303–305].

As SOS induces such notorious changes in the liver structure, disturbance of liver physiology is undoubtedly more profound and could have important implications in the organ's biological response to surgical and ischemic insult [139,297]. However, both the pathophysiology and the exact clinical consequences of SOS are still largely unknown. A recent meta-analysis and systematic review failed to show a significant link of SOS with posthepatectomy morbidity and mortality [306]. And furthermore, only one animal model has been validated of FOLFOX-related SOS [148].

Although sinusoidal endothelial cell (SEC) injury underlies the development of SOS, other mechanisms could be at play, including mitochondrial dysfunction. In fact, direct mitochondrial effect could be a relevant factor in oxaliplatin toxicity. This has been reviewed in **Chapter I** – "*Mitochondria and Liver Regeneration: Review of the Role of Energy Metabolism in the Pathophysiology of Posthepatectomy Liver Failure*". Meanwhile, bioenergetics dysfunction is a known factor in the development of posthepatectomy liver failure [19,146] (see also **Chapter II** – "*Mitochondrial Bioenergetics, Hepatic Pedicle Clamping and Posthepatectomy Liver Dysfunction*".

The research questions that prompted this Chapter were:

1. What are the prevalence, full pathological spectrum and clinical consequences of CALI, and SOS in particular, in patients undergoing liver resection for CRLM?
2. What is the role of bioenergetics in the pathogenesis of SOS-associated postoperative liver dysfunction after major hepatectomy with hepatic pedicle clamping?

Regarding the first query we performed a detailed review of the clinical and pathological data of a cohort of patients undergoing hepatectomy for CRLM in our department. And in order to address the second interrogation we attempted to replicate a previously validated oxaliplatin-induced animal model of SOS. In this model we then

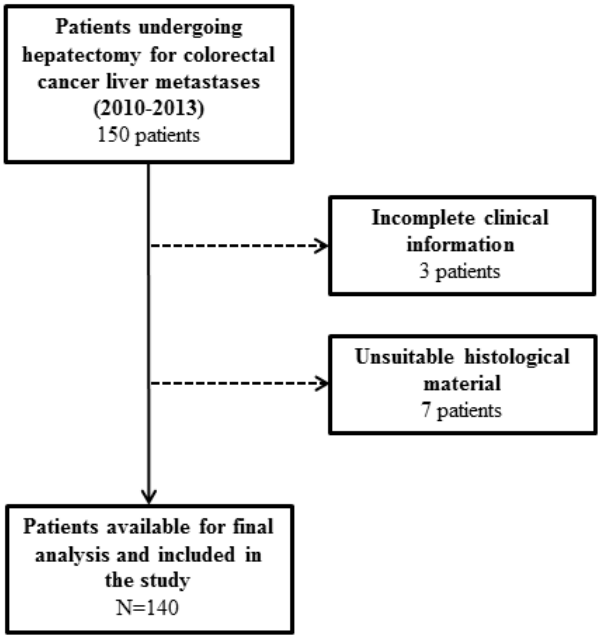
pursued to explore the potential contribution of mitochondrial dysfunction in the pathophysiology of chemotherapy-induced hepatotoxicity, in particular in the setting of major hepatectomy with hepatic pedicle clamping.

II. Material and Methods

A. Clinical study

This study consisted of clinical and pathological review of patients undergoing liver resection for CRLM in Serviço de Cirurgia A/III of Hospitais da Univerdade de Coimbra, Centro Hospitalar e Universitário de Coimbra (Head of Department: Prof. Doutor Francisco Castro e Sousa, MD, PhD) between January 2010 and July 2013. Exclusion criteria were poor histological material and/or insufficient clinical information (Figure 3.2). The study was approved by the institution's ethical committee and complied with the principles of the Declaration of Helsinki.

Figure 3.2 - Study population and exclusion criteria in the clinical study



1. Study population

The study included a total of 140 patients, 100 men and 40 women, with a mean age of 64±10 years (range 33-82); two thirds were older than 70 years. Twenty-seven (19%) patients suffered from diabetes mellitus. Primary tumor was located in the colon in 90 (64%) patients, in the rectum in 45 (32%) and in both sites in five (4%) patients. According to TNM classification, 91 (67%) patients were staged as node-positive (N+). Patients presented with a median of 2 liver metastases (range 1-16), with a mean diameter of 4.1±3.2cm (range 0.5-22) for the largest lesion. Sixty (43%) patients had a single nodule and 80 (57%) presented with multiple metastases. The location of the lesions was: right liver in 50 (36%) patients, left liver in 37 (26%) and bilobar in 53 (38%).

Clinical presentation of liver metastases was synchronous with the primary tumor in 74 (53%) patients and metachronous in 66 (47%). Among patients with synchronous disease, 12 (18.2%) underwent synchronous resection and in 54 (71.8%) the resection was metachronous: 44 underwent resection of the primary tumor before hepatectomy; while in 10 a “liver first” strategy was used; five of these were never operated on their primary lesion due to systemic tumor progression or major morbidity after hepatectomy. Furthermore, 26 (18.6%) cases were re-hepatectomies and 14 of those (10%) were two-stage hepatectomies, with an inter-stages median interval of 6 weeks (range 3-27).

2. Neoadjuvant chemotherapy

NCT was administered in 70 patients (50%); the remaining 70 patients (50%) formed the non-neoadjuvant chemotherapy (Non-NCT) group. Sixty-three (90%) patients received one line of chemotherapy and seven (10%) two. Chemotherapy regimens included 5-FU monotherapy in two (3%) patients, FOLFIRI in 48 (72%) patients and FOLFOX in 21 (31%). Twenty-nine (43%) patients were also treated with bevacizumab and 19 (28%) with cetuximab. A mean of 10.7±5.5 cycles (range 2-24) were administered. Thirty-eight (56%) patients received long-duration chemotherapy (more than nine cycles) while 32 (44%) received short-duration treatment (one to eight

cycles). The interval between the end of NCT and hepatectomy was six weeks for patients treated with bevacizumab and four weeks for all other agents.

3. Operative data

Hepatectomies were mostly performed through bilateral subcostal incisions, with routine use of intraoperative ultrasonography. Parenchymal transection was performed with ultrasonic dissection with CUSA™ Ultrasonic Surgical Aspirator (Integra, Plainsboro, N. USA) or Kelly-clamp crush technique. Hepatic inflow occlusion was used only when deemed necessary and in an intermittent strategy (15 minutes clamping with 5 minutes reperfusion) as previously reported [273]. Surgical procedures are summarized in Table 3.1. Minor resections were performed in 87 (62%) patients and major hepatectomy (resection of three or more Couinaud segments) in 53 (38%). Three patients (2%) underwent laparoscopic hepatectomy. Nineteen patients underwent additional portal vein embolization or ligation. Two (1%) were also treated with radiofrequency ablation. A mean of 346.5±855 mL of red blood cells were administered (range 0-5850). Hepatic pedicle clamping was performed in 98 patients, for a mean time of 28.2±23.2 minutes (range 0-104).

4. Postoperative course

Aminotransferase levels (AST and ALT), bilirubin and INR were collected on postoperative days one, three, five and seven. The median hospital length of stay (LOS) was 7 days (range 3-71). Postoperative morbidity was defined and graded up to the 90th postoperative day, according to Dindo et al [280]. Patients grading IIIA or higher were further classified as major morbidity. Posthepatectomy liver failure (PHLF) was defined by the “50-50 criteria”, according to Balzan et al [165] and graded according to Rahbari et al [162]. Bile leakage and postoperative hemorrhage were defined and graded according to published consensus definitions [162,281]. Liver-specific morbidity was defined as any complication directly related to liver resection, namely PHLF, bile leakage, biliary stenosis, ascites, bleeding or intra-abdominal abscess.

Table 3.1 - Description of hepatectomies performed in the clinical study (N= 140 patients undergoing hepatectomy for colorectal cancer liver metastases)

	N	%
Right Hepatectomy		
Classic	21	15
Extended	6	4.3
Classic + Left Atypical Resections	5	3.6
Extended + Left Atypical Resections	1	0.7
Left Hepatectomy		
Classic	4	2.9
Extended	3	2.1
Classic + Right Atypical Resections	1	0.7
Extended + Right Atypical Resections	2	1.4
Other anatomical resections		
Segmentectomy	19	13.6
Bisegmentectomy	14	10
Trisegmentectomy	3	2.1
Atypical resections		
< 4	24	17.1
≥ 4	9	6.4
Other anatomical resections + Atypical resections	28	20

5. Patient characteristics: chemotherapy vs. non-chemotherapy

Comparing the NCT Group with Non-NCT Group, there were no significant differences regarding age, sex and comorbidities. However, both the number (4.04 vs. 1.79, $p<0.001$) and diameter (4.63cm vs. 3.53cm, $p=0.045$) of hepatic lesions were higher in patients undergoing NCT. Also the proportion of cases with synchronous CRLM diagnosis (77.1% vs. 17.1%, $p<0.001$), major hepatectomies (48.6% vs. 27.1%, $p=0.014$) and two-stage hepatectomies (20% vs. 0%, $p<0.001$) was significantly larger in patients undergoing NCT when compared to those not treated with chemotherapy.

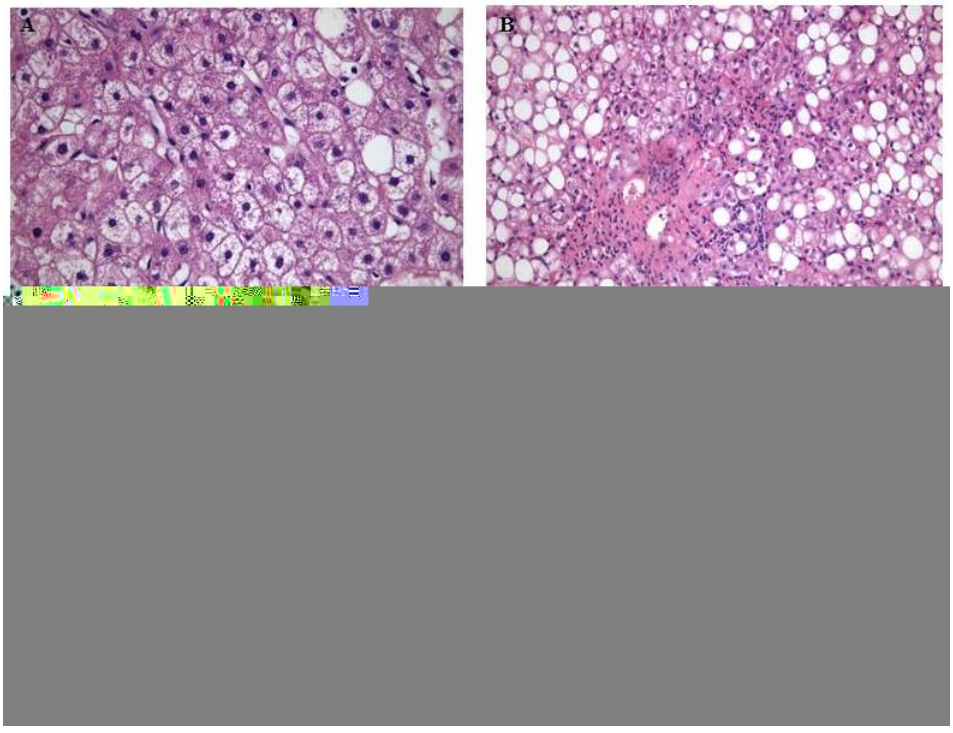
6. Pathological analysis

Archive pathological material from each patient was reviewed. A sample of non-tumoral liver parenchyma distant from the neoplasm (≥2cm) was examined and staining with hematoxylin and eosin (H&E), Masson's trichrome and reticulin was conducted. The histopathologic review was performed by two experienced hepatobiliary pathologists, blinded to patients' clinical data or outcome.

SOS-related lesions (Figure 3.3) were classified as follows: sinusoidal dilation, according to Rubbia-Brandt et al [136]: absent, mild (centrilobular involvement limited to less than one-third of lobular area), moderate (centrilobular involvement up to two-thirds of lobular area) and severe (complete lobular involvement); perisinusoidal hemorrhage, peliosis and necrosis: absent or present. Accordingly, we graded SOS as mild (in the presence of mild sinusoidal dilation without necrosis or peliosis) and moderate/severe (in the presence of mild sinusoidal dilation combined with necrosis, in the presence of moderate or severe sinusoidal dilation, or in the presence of peliosis).

Figure 3.3 - Representative samples of histological findings in the non-tumour bearing parenchyma in the clinical study (N=140 patients undergoing hepatectomy for colorectal cancer liver metastases)

(A) Ballooning degeneration, H&E, 400x; **(B)** Steatohepatitis, H&E, 200x; **(C)** Severe sinusoidal dilation with perisinusoidal hemorrhage, H&E, 100x; **(D)** Sinusoidal dilation, Masson's trichrome, 40x.



SH-related lesions (Figure 3.3) were classified according to Kleiner et al [307] as follows: steatosis (0-3): 0 for absent (less than 5% of hepatocytes involved), 1 for mild (5 to 33%), 2 for moderate (33% to 66%) and 3 for severe (more than 66%); lobular inflammation (0-3): 0 for absent, 1 for mild (less than two foci per 200x field), 2 for moderate (two to four foci per 200x field) and 3 for severe (more than 4 foci per 200x field); ballooning degeneration (0-2): 0 for absent, 1 for mild (few cells) and 2 for moderate and severe (many cells). A score from 0 to 8 was calculated for every patient and used to classify SH as it follows: absent (less than 3 points), borderline SH (3 or 4 points) and definite SH (more than 4 points) [307].

B. Experimental study

1. Study animals

Animals, 12 weeks old male Wistar rats (*Rattus norvegicus*) weighing 314 – 361 g, were purchased from Charles Rivers (Charles Rivers, France). Upon arrival animals were allowed to acclimatize and were housed in 12-hours light-dark cycles, with controlled temperature and humidity, with unlimited access to food and water. The study protocol was approved by the Animal Ethics Committee of the Faculty of Medicine of the University of Coimbra and all animals received care according to institutional guidelines.

2. Chemotherapy-induced liver injury model

For the induction of SOS we replicated the FOLFOX model described by Robinson et al [148], with a slight reduction in dosage based on our preliminary exploratory work (unpublished data) and literature review [308]. Briefly, intraperitoneal injection of oxalipatin (4 mg/kg) or vehicle (5% dextrose) was followed two hours later by intraperitoneal 5-fluorouracil (25 mg/kg) or vehicle (0.9% NaCl). Doses were given every week, during four weeks. In the end of the week 5, surgical procedures were performed. Four animals in the chemotherapy group succumbed to purulent peritonitis and were excluded from the final analysis.

All drugs were obtained in the Pharmaceutics Department of Centro Hospitalar e Universitário de Coimbra.

3. Surgical protocol

The surgical procedure was similar to that previously described in the Methods section of **Chapter II** - "*Mitochondrial Bioenergetics, Hepatic Pedicle Clamping and Posthepatectomy Liver Dysfunction*". In summary, under isoflurane anesthesia and after median laparotomy, a 70% hepatectomy with 30 minutes intermittent clamping (two periods of 15 minutes with 5-minute reperfusion) was performed, or sham procedure.

Animals (n=12) were divided in four groups:

- **Group 1** (n= 3) were injected with vehicle only and underwent sham laparotomy, isolation of hepatic pedicle and gentle liver manipulation, with the abdomen open during 35 minutes;
- **Group 2** (n= 3) were injected with vehicle only, underwent 70% hepatectomy with 30 minutes intermittent HPC;
- **Group 3** (n= 3) underwent FOLFOX injection and sham laparotomy, with isolation of hepatic pedicle and gentle liver manipulation, with the abdomen open during 35 minutes;
- **Group 4** (n= 3) underwent FOLFOX injection and 70% hepatectomy with 30 minutes intermittent HPC.

The abdomen was closed in all groups and the animals allowed to recover.

Animals were sacrificed at 12h after the surgical procedure, by craniocervical dissociation. Blood was collected from the inferior vena cava, and remnant liver (in groups 2 and 4) and whole liver (in groups 1 and 3), was weighed and removed.

4. Mitochondrial bioenergetics

a) Mitochondrial isolation

Mitochondria were isolated in homogenization medium containing 250 mM sucrose, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (pH 7.4), 0.5 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), and 0.1% fat-free bovine serum albumin, as previously described [69,276]. After homogenization of the minced blood-free hepatic tissue, the homogenate was centrifuged at 800 g for 10 min at 4º C. The supernatant was spun at 10,000 g for 10 min at 4º C to pellet mitochondria, which were re-suspended in a final washing medium. EGTA and bovine serum albumin were omitted from the final washing medium, adjusted at pH 7.4. Protein content was determined by the biuret method calibrated with bovine serum albumin.

b) Measurement of mitochondrial membrane potential

Mitochondrial membrane potential was estimated using an ion-selective electrode to measure the distribution of tetraphenylphosphonium (TPP^+). The voltage response of the TPP^+ electrode to log (TPP^+) was linear with a slope of 59 ± 1, in conformity with the Nernst equation. Reactions were carried out at 25º C, in a temperature-controlled water-jacketed chamber with magnetic stirring. Mitochondria (1 mg) were suspended in 1 ml of standard respiratory medium (130 mM sucrose, 50 mM KCl, 5 mM $MgCl_2$, 5mM KH_2PO_4, 50 µM ethylenediaminetetraacetic acid [EDTA], and 5 mM HEPES [pH 7.4] and 2 µM rotenone supplemented with 3 µM TPP^+). A matrix volume of 1.1 µl/mg protein was assumed.

The measured parameters were: membrane potential (mV); depolarization (mV); lag phase (seconds); and repolarization (mV). Readings were recorded in duplicate.

c) Measurement of oxygen consumption

Oxygen consumption of isolated mitochondria was polarographically determinated with a Clark oxygen electrode (Oxygraph, Hansatech Instruments Ltd,

UK) as described [69]. Mitochondria (1 mg) were suspended under constant stirring, at 25ºC, in 1.3 ml of standard respiratory medium (130 mM sucrose, 50 mM KCl, 5 mM MgCl2, 5mM KH_2PO_4, 50 µM EDTA, and 5 mM HEPES [pH 7.4] and 2 µM rotenone). State 3 respiration was induced by adding 200 nmol ADP. The oxygen consumption was also measured in the presence of 1 µM carbonylcyanide-p-trifluoromethoxyphenylhydrazon. State 3 and Respiratory Control Ratio (RCR) were calculated according to Chance and Williams [277].

d) Measurement of production of reactive oxygen species

Production of reactive oxygen species (ROS) was fluorometrically quantified using a PerkinElmer VICTOR3 plate-reader, at an excitation wavelength of 485 nm and an emission wavelength of 538 nm, respectively corresponding to the excitation and emission wavelengths of 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA) [309]. Isolated mitochondria (1 mg/mL) were suspended in standard respiratory medium and loaded with succinate 5 mM and H_2DCFDA 50 µM (prepared in dimethyl sulfoxide DMSO) for 15 min at 25 °C. 200 µL of the mitochondrial suspension was loaded into a 96-well plate and the fluorescence was monitored for 30 min to calculate the rate of ROS formation. The results were expressed as arbitrary relative fluorescence units.

e) Measurement of the mitochondrial permeability transition

Mitochondrial swelling was estimated by changes in light scattering, as monitored spectrophotometrically at 540 nm [310]. Reactions were carried out at 25 °C and measurement started with the addition of mitochondria (1 mg) to 2 mL of swelling medium (200 mM sucrose, 10 mM Tris–MOPS, 1 mM KH_2PO_4 and 10 µM EGTA pH 7.4) supplemented with 5 mM succinate and 2 µM rotenone. After a brief period for the recording of basal absorbance, different amounts of Ca2+ were added and the resulting alterations in light scattering were registered.

5. Blood biochemistry

Blood samples were processed in the laboratory for alanine aminotransferase (ALT), aspartate aminotransferase (AST) total bilirubin, gamma-glutamyltranspeptidase (GGT), International Normalized Ratio (INR), Tumour Necrosis Factor alpha (TNF-α) and glutamate dehydrogenase (GLDH). All analysis were performed with the Architect *c* System (Abbott Laboratories, USA).

6. Histology

Tissue samples analysis was performed on formalin-fixed paraffin-embedded tissue, using standard procedure: tissue samples were grossly inspected and sectioned, fixated in 4% formaldehyde, embedded in paraffin and cut in 4µm sections. Examination was performed on Haematoxylin and Eosin (H&E, Polysciences, Sakura Autostainer – Prisma 81D) stained slides observed in light microscope – Nikon Eclipse 50i, and images obtained using a Nikon-Digital Sight DS-Fi1 camera.

Sinusoidal obstruction syndrome (SOS) related lesions were classified according to Rubbia-Brandt et al: sinusoidal dilation – absent, mild (centrilobular involvement limited to less than one third of lobular area), moderate (centrilobular involvement up to two-thirds of lobular area) and severe (complete lobular involvement); perisinusoidal hemorrhage, peliosis and necrosis – absent or present.

7. Electron Microscopy

Liver biopsies were immediately fixated with glutaraldehyde (1.5% in cacodylate buffer 0.067 mol/L) and sliced in 1 mm^3 blocks for transmission electron microscopy (TEM). Afterwards the samples were washed with cacodylate buffer 0.2 mol/L and re-fixated in 1% osmium tetroxide in phosphate buffer for one hour. After being washed again in phosphate buffer, samples were ethanol-dehydrated and embedded in Epon. Ultra-thin sections (60 nm) were stained with uranyl and lead citrate. Samples were assessed by an experienced hepatobiliary and ultrastructural pathologist, blinded to the experimental groups. The following parameters were

investigated and graded as absent or present, according to Vreuls et al [311]: widening of the space of Disse; erythrocytes or blebs in the space of Disse; change in format of SEC's; decrease in SEC's organelles; intact cytoplasm of SEC's; and enlarged mitochondria in hepatocytes.

C. Statistical Analysis

Normality of distribution of continuous variables was assessed with Kolmogorov-Smirnov and Shapiro-Wilk tests, when indicated. Unless otherwise indicated, continuous variables with normal distribution were presented as mean ± standard deviation (SD); and variables with non-normal distribution as median and interquartile range (IQR). Mean values were compared using Student's *t* tests for variables with normal distribution and with Mann-Whitney *U* test for variables with non-normal distribution. Categorical variables were described by absolute and relative frequencies, and the distributions were compared using Chi squared tests. A two-sided *p* value of ≤0.05 was considered to indicate statistical significance. A binary logistic regression was conducted to examine the individual effect of each parameter on postoperative morbidity and mortality. SPSS™ (version 21.0, SPSS inc., Chicago, IL.) was used for statistical calculations.

III. Results

A. Clinical Study

1. Postoperative morbidity and mortality

Postoperative complications were present in 31 (22.1%) patients. Non-liver-specific morbidity was reported in 7 (5%) patients. Liver-specific complications, were present in 25 (17.9%) patients: biloma or intra-abdominal abscess in seven patients, biliary fistula in five, biliary stenosis in two, liver hemorrhage in two, and PHLF in nine (6.4%) patients, of which two were grade A (1.4%), two were grade B (1.4%) and five

grade C (3.6%). Twenty-one (15%) patients presented major morbidity. Six (4.3%) postoperative deaths were reported, five of which due to PHLF grade C.

2. Prevalence and patterns of histologic liver injury

In the study population, steatosis was present in 47 patients (34%), of which 15 were moderate or severe steatosis (11%) and 23 steatohepatitis (16%). Sinusoidal dilation was present in approximately half of the study population, 73 patients (52%). Moderate or severe SOS was prevalent in 18 patients (13%), while peliosis was present in six (4%).

3. Impact of neoadjuvant chemotherapy on liver injury

Patients who underwent NCT showed a higher incidence of peliosis (25.7% vs. 11.6%, OR 1.094, CI 95% 1.018-1.175, p=0.049) and moderate and severe SOS (21.4% vs. 4.3%, OR 6.091, CI 95% 1.677-22.124, p=0.004), when compared with the non-NCT group. The chemotherapy agent (oxaliplatin or irinotecan) did not significantly correlate with any particular histological pattern. However, bevacizumab offered significant protection against severe and moderate SOS. Only three (10.3%) patients treated with bevacizumab presented with moderate and severe SOS, against 12 (31.6%) not treated with this agent (OR 0.25, CI 95% 0.063-0.991, p=0.045). Longer NCT (more than 9 cycles) was not associated with more liver injury.

4. Impact of co-morbidities on liver injury

Diabetic patients had significantly higher incidence of both steatosis (59.3% vs. 27.4%, OR 3.848, CI 95% 1.609-9.200, p=0.003) and moderate and severe steatosis (25.9% vs. 7.1%, OR 4.594, CI 95% 1.497-14.099, p=0.01). However, there was a reduced risk for sinusoidal dilation (33.3% vs. 56.6%, OR 0.383, CI 95% 0.158-0.925, p=0.034).

5. Impact of neoadjuvant chemotherapy on morbidity and mortality

A statistically significant association between the administration of NCT and postoperative complications or mortality was not found. Mean LOS was also similar between the two groups (NCT group 10.9±11.8 vs. non-NCT group 10.6±10.2, p=0.855). The number of NCT cycles did not correlate either with postoperative morbidity or mortality, or with a higher LOS (<9 cycles 12.0±13.3 vs. ≥9 cycles 10.5±10.9, p=0.605).

6. Impact of liver injury on morbidity and mortality

Steatohepatitis-related lesions

The impact of SH-related lesions on morbidity and mortality is shown in Table 3.2. Steatosis reduced the risk of postoperative complications (10.6% vs. 28%, p=0.014) and major morbidity (6.4% vs. 19.4%, p=0.047). Moderate and severe steatosis related with the absence of postoperative complications (0% vs. 24.8%, p=0.019). The presence of SH reduced the incidence of overall morbidity (4.3% vs. 25.6%, p=0.016).

SOS-lesions

The impact of SOS-related lesions on morbidity and mortality is shown in Table 3.2. The presence of sinusoidal dilation increased the risk of overall morbidity (31.5% vs. 12%, p=0.008), liver-specific complications (26% vs. 9%, p=0.014) and major morbidity (22% vs. 7.5%, p=0.019). Peliosis was a risk factor for PHLF (50% vs. 4.5%, p=0.003), major morbidity (50% vs. 12.9%, p=0.044) and mortality (50% vs. 2.2%, p=0.001). Patients with moderate and severe SOS had a higher incidence of postoperative complications (44.4% vs. 18.9%, p=0.029), PHLF (27.8% vs. 3.3%, p=0.002), liver-specific morbidity (38.9% vs. 14.8%, p=0.021), major complications (33.3% vs. 12.3%, p=0.031) and mortality (16.7% vs. 2.5%, p=0.028).

Table 3.2 - Impact of liver injury on morbidity and mortality (univariate analysis) in the clinical study (N= 140 patients undergoing hepatectomy for colorectal cancer liver metastases)
PHLF – Posthepatectomy Liver Failure; CASH – Chemotherapy-Associated Steatohepatitis; SOS –Sinusoidal Obstruction Syndrome

	Morbidity	***Liver-specific Morbidity***	***PHLF***	***Major Morbidity***	***Mortality***
Steatosis (n=47)	**5 (10.6%)**	4 (8.5%)	1 (2.1%)	**3 (6.4%)**	1 (2.1%)
No Steatosis (n=93)	**26 (28%)**	21 (22.6%)	8 (8.6%)	**18 (19.4%)**	5 (5.4%)
OR	**0.307**	0.319	0.231	**0.284**	0.383
CI 95%	**0.109-0.861**	0.103-0.991	0.028-1.904	**0.079-1.019**	0.43-3.373
p	**0.014**	0.06	0.272	**0.047**	0.664
Moderate and Severe Steatosis (n=15)	**0 (0%)**	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Mild or No Steatosis (n=125)	**31 (24.8%)**	25 (20%)	9 (7.2%)	21 (16.8%)	6 (4.8%)
OR	**0.862**	0.87	0.885	0.874	0.888
CI 95%	**0.800-0.930**	0.810-0.933	0.833-0.942	0.816-0.936	0.836-0.943
p	**0.019**	0.073	0.597	0.127	1
CASH (n=23)	**1 (4.3%)**	1 (4.3%)	0 (0%)	1 (4.3%)	0 (0%)
No CASH (n=117)	**30 (25.6%)**	24 (20.5%)	9 (7.7%)	20 (17%)	6 (5.1%)
OR	**0.132**	0.176	0.824	0.22	0.828
CI 95%	**0.017-1.020**	0.023-1.373	0.762-0.892	0.028-1.731	0.767-0.895
p	**0.016**	0.077	0.355	0.198	0.589
Sinusoidal Dilation (n=73)	**23 (31.5%)**	**19 (26%)**	7 (9.6%)	**16 (22%)**	4 (5.5%)
No Sinusoidal Dilation (n=67)	**8 (12%)**	**6 (9%)**	2 (3%)	**5 (7.5%)**	4 (6%)
OR	**3.393**	**3.577**	3.447	**3.481**	1.884
CI 95%	**1.395-8.247**	**1.332-9.610**	0.690-17.216	**1.198-10.114**	0.334-10.636
p	**0.008**	**0.014**	0.169	**0.019**	0.682
Peliosis (n=6)	3 (50%)	3 (50%)	**3 (50%)**	**3 (50%)**	**3 (50%)**
No Peliosis (n=134)	28 (20.9%)	22 (16.4%)	**6 (4.5%)**	**18 (12.9%)**	**3 (2.2%)**
OR	3.786	5.091	**21.333**	**6.444**	**43.667**
CI 95%	0.724-19.783	0.964-26.890	**3.535-128.745**	**1.206-34.425**	**6.105-312.316**
p	0.122	0.07	**0.003**	**0.044**	**0.001**
Moderate and Severe SOS (n=18)	**8 (44.4%)**	**7 (38.9%)**	**5 (27.8%)**	**6 (33.3%)**	**3 (16.7%)**
Mild or No SOS (n=122)	**23 (18.9%)**	**18 (14.8%)**	**4 (3.3%)**	**15 (12.3%)**	**3 (2.5%)**
OR	**3.443**	**3.677**	**11.346**	**3.567**	**7.933**
CI 95%	**1.224-9.689**	**1.259-10.736**	**2.704-47.608**	**1.165-10.921**	**1.467-42.909**
p	**0.029**	**0.021**	**0.002**	**0.031**	**0.028**

However, patients with moderate and severe SOS underwent significantly more major hepatectomies (72.2% vs. 32.8%, OR 5.33, CI 95% 1.777-15.988, p=0.003) and longer periods of HPC (40.24±25.03 vs. 26.33±22.45, p=0.021). The number of nodules was also higher in the patients with moderate and severe SOS (4.17±3.808 vs. 2.72±2.764, p=0.051). This prompted the performance of a multivariate analysis.

Table 3.3 - Multivariate analysis of risk factors for postoperative morbidity in the clinical study (N= 140 patients undergoing hepatectomy for colorectal cancer liver metastases)
PHLF – Posthepatectomy Liver Failure; SOS – Sinusoidal Obstruction Syndrome; CASH – Chemotherapy-Associated Steatohepatitis

	Morbidity	Liver-specific Morbidity	PHLF	Major Morbidity	Mortality
Sinusoidal Dilation					
p	**0.020**	**0.016**	0.811	0.056	0.497
OR	**3.884**	**4.921**	0.751	3.690	0.383
CI 95%	**1.233-12.232**	**1.346-17.995**	0.072-7.838	0.968-14.072	0.024-6.084
Fibrosis					
p	0.317	0.254	0.606	0.423	0.199
OR	1.808	2.046	1.613	1.731	4.626
CI 95%	0.567-5.736	0.599-6.991	0.262-9.946	0.453-6.620	0.447-47.850
Moderate and Severe SOS					
p	0.848	0.753	0.141	0.975	0.586
OR	1.145	0.791	4.859	0.975	2.191
CI 95%	0.286-4.592	0.184-3.409	0.593-39.817	0.200-4.767	0.130-37.005
Steatosis					
p	0.393	0.282	0.915	0.447	0.965
OR	0.521	0.377	1.145	0.499	0.942
CI 95%	0.117-2.325	0.064-2.229	0.095-13.753	0.083-2.997	0.065-13.589
CASH					
p	0.307	0.604	0.998	0.651	0.998
OR	0.272	0.494	-	0.539	-
CI 95%	0.022-3.301	0.034-7.122	-	0.037-7.875	-
Hepatic Pedicle Clamping					
p	0.967	0.894	0.626	0.513	0.903
OR	0.976	0.920	0.636	1.604	1.162
CI 95%	0.315-3.023	0.270-3.130	0.103-3.923	0.389-6.624	0.105-12.874
Major Hepatectomy					
p	0.268	0.093	0.184	0.086	0.208
OR	1.747	2.511	3.333	2.786	4.541
CI 95%	0.652-4.685	0.859-7.345	0.565-19.663	0.863-8.992	0.431-47.818

7. Multivariate analysis

On multivariate analysis (Table 3.3) sinusoidal dilation was found to be an independent risk factor for postoperative morbidity, increasing almost four times the risk of overall complications (p=0.02, OR 3.884, CI 95% 1.233-12.232) and five times the risk of liver-specific complications (p=0.016, OR 4.921, CI 95% 1.346-17.995). However, no effect was found on the incidence of PHLF, major morbidity or mortality

B. Experimental study

1. Induction of sinusoidal obstruction syndrome with chemotherapy

No significant effect of FOLFOX was observed on H&E, including sinusoidal dilation, hepatocellular necrosis or atrophy in chemotherapy-treated versus vehicle-treated animals. There was also no widening or increased content in the space of Disse and no injury to endothelial cells on TEM.

2. Effect of chemotherapy on mitochondrial energetics and structure

Animals in groups 3 and 4 (chemotherapy-treated) presented longer time to re-energize the mitochondrial membrane potential after complete phosphorylation of ADP into ATP (lag phase 13.4 ±1.9 seconds) versus animals in groups 1 and 2 (lag phase 10.2 ±1.1 seconds) (p=0.012) (Figure 3.4).

Increased-size mitochondria in hepatocytes were described more frequently on TEM in chemotherapy-treated animals (5 out of 6) versus control animals (1 out of 6) (p=0.021) (Figure 3.5). No statistically significant change was observed in the other bioenergetics variables, including radical oxygen species and mitochondrial permeability transition.

Figure 3.4 - Effect of chemotherapy on mitochondrial function in the experimental study (N= 12 Wistar rats undergoing oxaliplatin and 5-fluorouracil or vehicle injection for four weeks)

Animals treated with chemotherapy (groups 3 and 4) displayed a longer lag phase, i.e. the time to re-energize the mitochondrial membrane potential after complete phosphorylation of ADP into ATP (13.4 ±1.9 seconds) versus vehicle-treated animals (groups 1 and 2) (lag phase 10.2 ±1.1 seconds) (p=0.012)

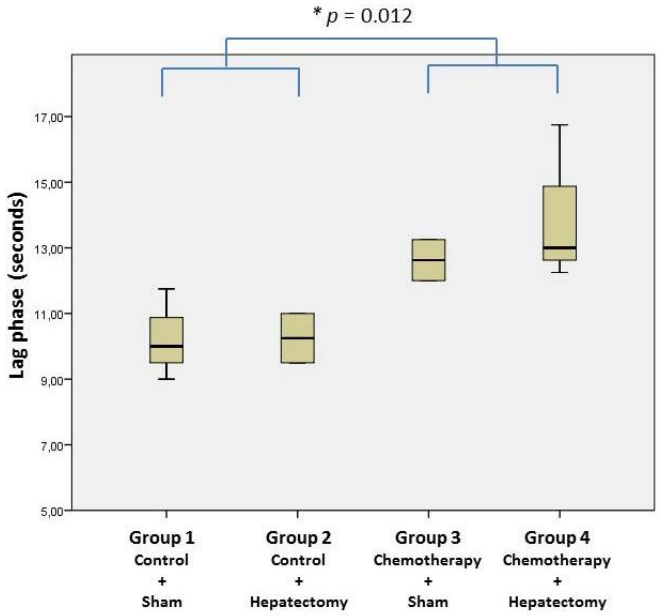
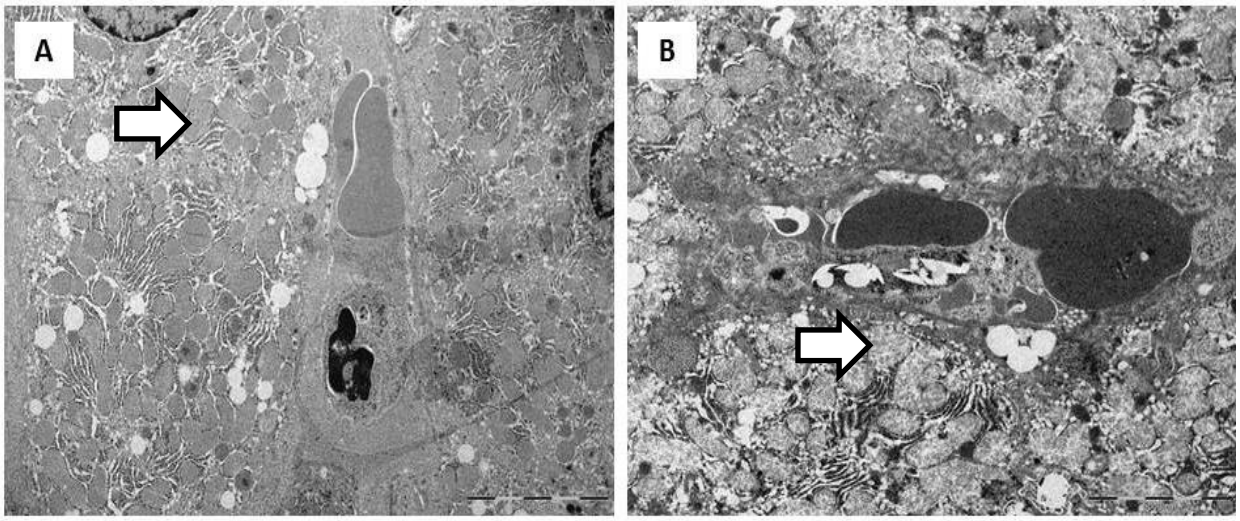


Figure 3.5 - Transmission electron microscopy in the experimental study (N= 12 Wistar rats undergoing oxaliplatin and 5-fluoruracil or vehicle injection for four weeks)

A. Hepatocytes with normal-sized mitochondrial in the vehicle-treated animals (n=6) (white arrow)
B. Hepatocytes with swelled mitochondria in the chemotherapy-treated animals (n=6) (white arrow)



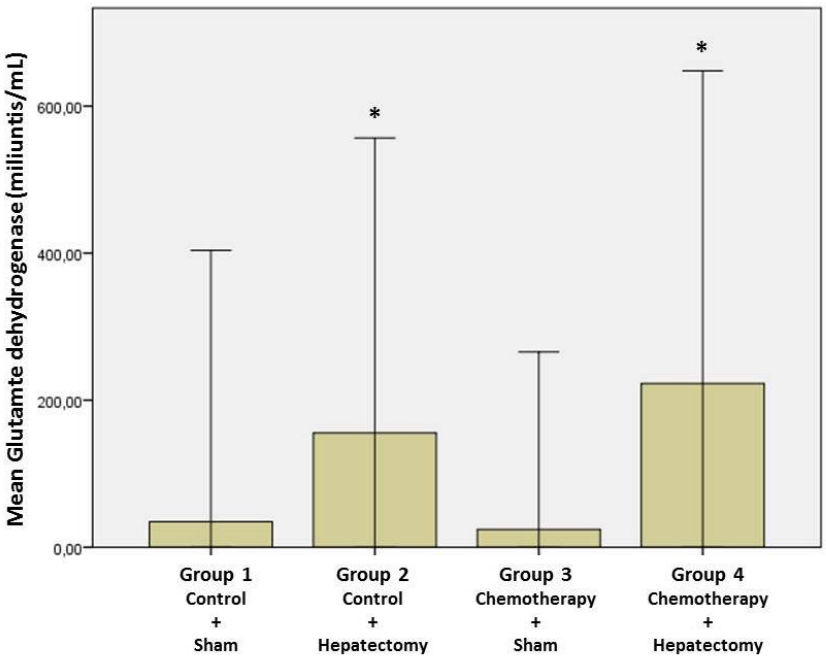
3. Effect of hepatectomy with hepatic pedicle clamping on mitochondrial energetics and hepatocellular function

Posthepatectomy liver mitochondrial function was slightly hampered. Animals in groups 2 and 4 demonstrated lower mitochondrial membrane potential (209.3 ±1.6 mV) versus animals in groups 1 and 3 (211.7 ±1.5 mV) (p=0.045). Also glutamate dehydrogenase, a marker of mitochondrial dysfunction, was increased in groups 2 and 4 taken together (134 miliunits/mL IQR 71 – 356) versus sham-operated animals (24.4 miliunits/mL IQR 5 – 58) (p=0.033) (Figure 3.6).

No statistically significant change was observed in the other bioenergetics variables, including radical oxygen species and mitochondrial permeability transition.

Figure 3.6 - Noninvasive marker of mitochondrial dysfunction after hepatectomy with hepatic pedicle clamping in the experimental study (N= 12 Wistar rats undergoing oxaliplatin and 5-fluorouracil or vehicle injection for four weeks)

Glutamate dehydrogenase was increased in animals undergoing hepatectomy with hepatic pedicle clamping (groups 2 and 4) taken together (134 miliunits/mL IQR 71 – 356) versus sham-operated animals (groups 1 and 3) (24.4 miliunits/mL IQR 5 – 58) (* p=0.033)



Hepatectomy with HPC was associated with increased markers of hepatocellular necrosis (ALT 1595 UI/L IQR 997 – 3652; AST 1810 IQR 1045 – 4190) versus sham-operated animals (ALT 119 IQR 80 – 302; AST 275 IQR 249 – 944) (p=0.006 and

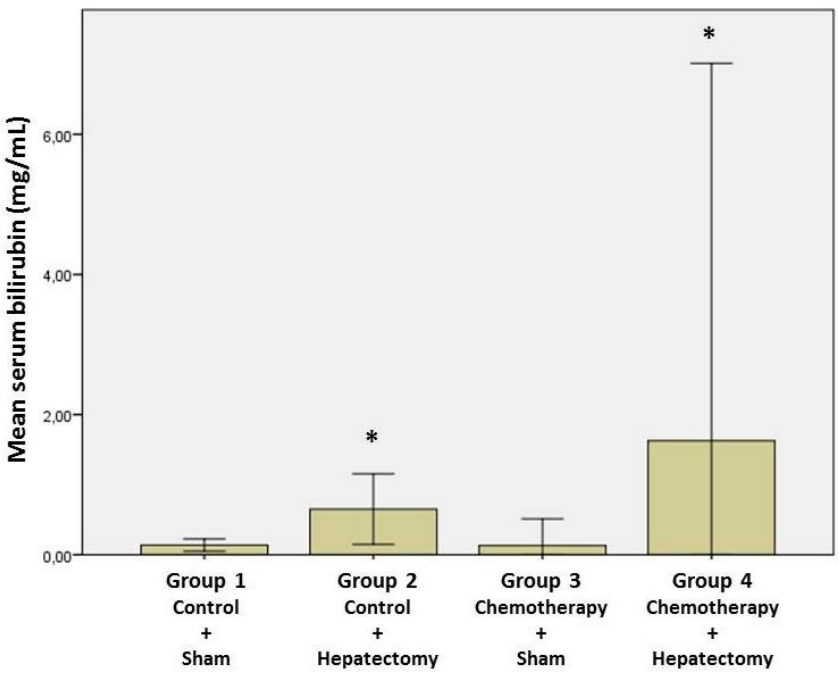
p=0.018, respectively). Also hepatocellular function was decreased in groups 2 and 4, with higher peak bilirubin (1.1 mg/dL IQR 0.4 – 1.7) than sham operated animals (0.14 mg/dL IQR 0.1 – 0.17) (p=0.006) (Figure 3.7). No statistically significant difference in these markers was observed in chemotherapy-treated versus vehicle-treated animals, however. Furthermore, there was no statistically significant change in the levels of TNF-α in either group.

4. Correlation of mitochondrial dysfunction with postoperative hepatocellular function

In all the study animals mitochondrial membrane potential inversely correlated with ALT (r= -0.756; p=0.011) and AST (r= -0.770; p=0.009). Furthermore, GLDH strongly correlated with bilirubin levels (r=0.801; p=0.005).

Figure 3.7 - Markers of hepatocellular function in the experimental study (N= 12 Wistar rats undergoing oxaliplatin and 5-fluoruracil or vehicle injection for four weeks)

Peak serum bilirubin was higher (1.1 mg/dL IQR 0.4 – 1.7) in animals undergoing 70% hepatectomy with hpatic pedicle clamping (groups 2 and 4) versus sham operated animals (groups 1 and 3) (0.14 mg/dL IQR 0.1 – 0.17) (*p=0.006)



IV. Discussion

In this Chapter two distinct investigation questions were formulated. The first question concerned the association of CALI with posthepatectomy liver dysfunction, while the second question dwelled on the involvement of mitochondrial dysfunction in its pathogenesis.

The impact of neoadjuvant chemotherapy on the liver parenchyma and associated posthepatectomy morbidity and mortality has been the subject of intense research. Previous studies have already established an association between NCT and liver injury, such as SOS and SH. [116,136,138,295,296] However, the impact of both the chemotherapy itself and the drug-induced liver lesions on postoperative morbidity remains controversial. These interrogations prompted a review of clinical and pathological data of patients undergoing hepatectomy for CRLM.

In the clinical study we failed to confirm the deleterious effect of high number of cycles of NCT on postoperative morbidity. Other studies have proved a direct relationship between NCT and morbidity [133], while others have reported findings similar to our results [116,138]. The only prospective randomized trial of NCT reported a higher incidence of postoperative complications in patients that underwent NCT [130].

We then investigated the patterns of liver injury and its consequences. Vauthey et al reported a higher incidence of SH in patients treated with irinotecan-based chemotherapy [116]. Reissfelder et al further confirmed this, but the type of NCT was not differentiated [312]. Steatosis was found in 34% and steatohepatitis in 16% of the study population but they were unrelated to chemotherapy in our study. Furthermore, unlike what is described by Vauthey et al [116], SH did not increase postoperative mortality. In fact, although the multivariate analysis failed to confirm it, both steatosis and SH were associated with decreased postoperative morbidity. Interestingly, Viganò et al had previously correlated steatosis with a better long-term prognosis [313].

Sinusoidal dilation was the most prevalent pattern of injury, present in half of the patients, while moderate and severe SOS was present in 13% of patients. Univariate

analysis revealed that patients undergoing NCT had a higher incidence of sinusoidal dilation, peliosis and moderate and severe SOS. Rubbia-Brandt et al were the first to report an association between oxaliplatin-based NCT and sinusoidal dilation [295] and other studies have confirmed this fining [136,138,296]. However, we did not find a significant association of any NCT agent or combination with SOS. Nonetheless, bevacizumab-treated patients had a lower incidence of moderate and severe SOS (p=0.045). This protective effect of bevacizumab was first reported by Rubbia-Brandt et al [136] and was later confirmed in other studies [294,296]. Surprisingly, in our study, diabetic patients had lower incidence of sinusoidal dilation (p=0.034), even though the multivariate analysis failed to confirm this finding. To our knowledge, only one study has reported this finding before [314].

SOS, formerly known as veno-occlusive disease or Stuart-Bras syndrome [137], is particularly relevant in liver surgery as it can increase perioperative bleeding [138], postoperative morbidity [138,141] and decrease tolerance to HPC [139]. It also lowers regenerative capacity, increases risk of postoperative liver failure [142] and can be associated with decreased overall survival [315]. However, there is still no consensual classification for SOS, as several lesions, such as sinusoidal dilation, perisinusoidal hemorrhage, fibrosis and nodular regenerative hyperplasia, are included in this broad spectrum.

In our series SOS significantly increased postoperative morbidity. Nevertheless potential bias may have influenced this result, as patients with moderate and severe SOS had higher tumor load, underwent major hepatectomies more often and had longer periods of HPC. However, the multivariate analysis confirmed sinusoidal dilation as an independent major risk factor for overall morbidity (OR 3.884; p=0.02) and liver-specific complications (OR 4.921; p=0.016). As such, the results of the present clinical study are in keeping with the previous reports of increased postoperative morbidity associated with SOS. However, a definitive association of SOS with PHLF, major morbidity or mortality was not demonstrated on multivariate analysis. This is also in agreement with a recently published meta-analysis and systematic review on the subject [306].

Having addressed the first investigation question, a thorough reflection on the pathophysiology of chemotherapy-induced SOS, as well as its effects on liver regeneration, are in order before addressing the second query. Sinusoidal endothelial cell injury plays a pivotal role in the pathogenesis of SOS, possibly due to glutathione depletion [137], emphasizing the potential role of oxidative stress [316,317]. Other putative mechanisms involved in the pathogenesis of SOS include disturbances in angiogenesis, coagulation, and cell adhesion. These are suggested by the protective role of aspirin and bevacizumab [136,296,318–320], which was also confirmed in our clinical study. Portal hypertension is also a common finding in SOS, as is reflected by an increase in spleen size and decreased platelet count [297,303].

Current knowledge on the pathophysiology of SOS largely relies on the work of DeLeve et al. The authors developed the cardinal experimental model of SOS after exposure to the pyrrolizidine alkaloid monocrotaline (MCT), replicating the histologic and ultrastructural findings of the disease, including SEC injury and detachment, as well as destruction of the sinusoidal wall [321]. Injury progression leads to formation of gaps in the sinusoidal membrane, leakage of blood cells into the space of Disse, hepatocellular necrosis and deposition of extracellular matrix. Similar findings on TEM were also observed by Vreus et al in human samples [311].

In attempts to investigate the clinical consequences of SOS in liver surgery, several studies have confirmed that MCT-induced SOS decreases liver regeneration, hampers liver function and increases susceptibility to ischemia-reperfusion injury in animal models of major hepatectomy with or without hepatic pedicle clamping [147,322].

However, experimental evidence for a direct deleterious effect of chemotherapy on liver regeneration is conflicting. Rickenbacher et al investigated the effect of exposure to different chemotherapy regimens on liver regeneration after 75% hepatectomy in C57BL/6 mice [323]. After administration on five consecutive days of several chemotherapy combinations (including FOLFIRI and FOLFOX), animals underwent 75% hepatectomy and regeneration was assessed with PCNA and Ki-67. No deleterious effect of chemotherapy was noted and the authors concluded that chemotherapy had no toxic effect on liver regeneration after major hepatectomy.

Another study reported different findings after single dose injection of oxaliplatin, oxaliplatin plus 5-FU and bevacizumab, followed three days later by 70% hepatectomy. The authors report a decrease in bromodeoxyuridine positive nuclei and liver regrowth ratio [324]. These results must be interpreted with caution, however. Of note, no histological injury was present, possibly due to the short-term exposure to the drugs. Also, in these studies hepatectomy was performed without hepatic pedicle clamping, a known co-factor in hepatocellular injury.

Furthermore, all the aforementioned hepatotoxicity models rely on acute exposure and acute toxic effects, unlike what is witnessed in the clinical setting with chemotherapy-associated liver injury, in which longer exposure is the rule. A major breakthrough in this field was provided by Robinson et al, with the publication of a murine model of oxaliplatin-induced SOS [148]. The authors injected C57Bl/6 mice for five weeks with oxaliplatin and 5-FU and describe mild sinusoidal dilation, SEC disruption and glutathione depletion. As such, we decided to use this model, with slight modifications based on our preliminary works, especially in dose adjustment for decreased mortality (unpublished data), and on literature review [308].

Although most chronic liver diseases are associated with some degree of mitochondrial dysfunction [54], this remains unproven in CALI and SOS. However, this link is suggested by indirect data. As reviewed in **Chapter I** - "*Mitochondria and Liver Regeneration: Review of the Role of Energy Metabolism in the Pathophysiology of Posthepatectomy Liver Failure*", oxaliplatin is directly toxic to mitochondria and can hamper cellular respiration. A link with mitochondrial toxicity is also suggested by another study of Robinson et al [325]. The authors describe an association of increased expression of copper transporter ATP7B in tissue resistance to oxaliplatin. This is easily explained as copper metabolism parallels that of platinum compounds [326]. Intriguingly, defects of ATP7B have been linked to decreased copper excretion and to a Wilson's disease-like phenotype in animal models, characterized by mitochondrial dysfunction and acute liver dysfunction [327]. Finally, Kim et al noticed an increase in glucose uptake on 18F-fluorodeoxyglucose positron emission tomography in the liver parenchyma in patients with SOS after platinum-based chemotherapy [328]. This could suggest an increase in the glycolytic pathway for the production of energy, to which healthy liver cells only resort in the setting of failure of mitochondrial oxidative

phosphorylation [329]. One of the putative mechanisms of oxaliplatin-induced mitochondrial injury is damage to mitochondrial DNA (MtDNA). Oxaliplatin forms DNA adducts and MtDNA lacks protective histones and is more susceptible to damage than the nuclear genome [31]. Since MtDNA contains 13 genes that code for important protein subunits of the electron transport chain, hampered cellular bioenergetics could be an early event in oxaliplatin-toxicity.

Another possible pathway for bioenergetics derangement in SOS is through disturbances in microcirculatory flow caused by the SEC injury and detachment. This could lead to decreased hepatic oxygen delivery and subsequent energetic dysfunction of liver cells [330]. In fact, an increased collagen deposition in the space of Disse is a hallmark of SOS, causing sinusoidal capillarization, a finding noted by Narita et al to be associated with decreased indocyanine green excretion [331]. And Jafari et al describe a decrease in parenchymal oxygenation in rats with MCT-induced SOS [322].

Whether by direct mitochondrial toxicity or by decreased parenchymal oxygenation, bioenergetics derangement in SOS is a definite possibility. This postulate led us to seek the answer for the second research question in this Chapter by performing an experimental study using the Robinson et al FOLFOX-induced model. In this study, we were able to demonstrate a modest yet significant effect of oxaliplatin on liver energy metabolism. Mitochondrial lag phase, i.e. the time needed to repolarize the mitochondrial membrane potential after complete ADP phosphorylation, was significantly longer in chemotherapy-treated animals. This could result from a selective impairment of the Adenine Nucleotide Translocator (ANT), the transmembrane complex that exchanges ADP for ATP. Interaction with the ANT carrier has already been described in isolated mitochondrial models of xenobiotic toxicity and DILI [332,333]. ANT is also a component of the mitochondrial permeability transition (MPT) pore, a non-selective channel in the mitochondrial outer membrane. The MPT causes mitochondrial swelling and allows the leakage of protons, with subsequent delayed phosphorylation and reduced energy efficiency. Cytochrome c is also released into the cytoplasm, activating the caspase-3 cell death pathway [33]. In fact we observed an increase in mitochondria size on TEM in chemotherapy-treated animals, suggesting mitochondrial swelling and the development of MPT.

However, chemotherapy-treated animals did not present any relevant curtailment of liver function, nor increased markers of hepatocellular necrosis. Also of note was the absence of an increased production of ROS, which is in apparent contradiction with the previous reports of oxidative stress in the pathogenesis of oxaliplatin toxicity [316].

Furthermore, sinusoidal dilation and SEC injury, the histologic hallmarks of SOS, were not replicated in the chemotherapy-treated group. And neither TEM was able to display more subtle ultrastructural changes in SEC's. The absence of significant parenchymal damage on histology suggests that microcirculatory derangement is a less important contributory factor and could be an argument for direct mitochondrial toxicity of oxaliplatin, but this remains unproven. Furthermore, a detailed analysis of the Robinson et al models reveals that the authors only reported mild sinusoidal dilation in their FOLFOX-model, unlike the MCT model where the complete pathologic spectrum of SOS is found. And very recently, Lentschener et al have reported that they too were unable to replicate Robinson's FOLFOX-induced SOS model [334]. Further investigation in this arena is obviously needed.

Other significant findings emerge from the experimental study. In our model hepatectomy with HPC was associated with worse mitochondrial membrane potential. This is congruent with our previous results, both in the clinical and experimental setting, whereby HPC was associated with a derangement of mitochondrial oxidative phosphorylation (see **Chapter II** - *"Mitochondrial Bioenergetics, Hepatic Pedicle Clamping and Posthepatectomy Liver Dysfunction*"). Also in keeping with our previous clinical study [19], there was a strong and significant correlation between mitochondrial dysfunction and posthepatectomy levels of ALT and AST. Although aminotransferases are not a surrogate marker of outcome, they are a sensitive marker of hepatocellular ischemic injury and necrosis [288]. And interestingly, there was a very strong and highly significant correlation between glutamate dehydrogenase, a non-invasive marker of mitochondrial dysfunction, and bilirubin, one of the most clinically useful markers of posthepatectomy liver function. We postulate that GLDH could become a sensitive and specific marker of posthepatectomy liver outcome, much as it is in the setting of drug-induced liver injury [187] (see also **Chapter I** – "*Mitochondria and Liver Regeneration: Review of the Role of Energy Metabolism in the Pathophysiology of Posthepatectomy Liver Failure"*).

In conclusion, although preoperative chemotherapy is a powerful tool for the multidisciplinary management of CRLM, it can also severely injure the liver parenchyma. In keeping with other reports, steatosis was not associated with increased morbidity and sinusoidal injury was the prevalent histologic pattern. In fact, severe sinusoidal injury was associated with an increased incidence of PHLF and SOS was an independent risk factor for liver-specific morbidity. Whether because of sinusoidal endothelial cell injury and subsequent disturbance of microcirculatory flow, or by direct mitochondrial toxicity of oxaliplatin, bioenergetics failure possibly plays a key role in the hepatocellular dysfunction and morbidity associated with hepatectomy in SOS.

Meanwhile, in the clinical setting preoperative identification of patients with established SOS is of paramount importance, since surgical strategy could be modified accordingly. In fact, a higher threshold for future liver remnant volume in CALI has been put forward by Narita et al [140] and is an argument for the use of parenchymal modulating therapies, such as portal vein embolization. And ultimately, advances in pharmacogenomics could allow targeted selection of chemotherapeutic agents, maximizing its effect while minimizing toxicity [335].

Although there is a suggestion for the definite involvement of mitochondrial dysfunction in the pathogenesis of CALI-induced posthepatectomy morbidity, many questions remain unanswered, especially regarding the exact molecular mechanisms of oxaliplatin-induced injury. Furthermore, the intriguing observation of decreased prevalence of SOS in diabetic patient cannot be easily explained and merits further investigation.

Given the diverse and complex putative pathways involved, the pathophysiology of SOS remains elusive. While hepatocyte and SEC co-cultures could provide the ideal experimental model to investigate the role of energy metabolism in CALI [336,337], further research will hopefully shed light on this complex liver disorder, as well as on its consequences on the liver’s response to surgical aggression.

Chapter IV - Bioenergetic Adaptations of the Liver in the ALPPS Procedure: How Liver Regeneration Correlates with Mitochondrial Energy Status

Part of this chapter has been submitted as:
Bioenergetic Adaptations of the Liver in the ALPPS Procedure – How Liver Regeneration Correlates with Mitochondrial Energy Status
Alexandrino H, Rolo A, Teodoro JS, Donato H, Martins R, Serôdio M, Martins M , Tralhão JG, Caseiro Alves F, Palmeira C, Castro e Sousa
HPB – Journal of the Hepato-Pancreato-Biliary Association (submitted)

I. Introduction

Liver resection is only limited by the capacity of the liver to recuperate lost mass, which is dependent upon the size and quality of the future liver remnant (FLR) [7]. The Associating Liver Partition and Portal Vein Ligation for Staged Hepatectomy, or ALPPS, is a recently developed two-stage procedure that allows the performance of extended resections while significantly increasing the volume of the FLR between stages in an otherwise unresectable patient [249]. However, it is also associated with increased morbidity and up to 12% mortality rate; mostly due to Posthepatectomy Liver Failure (PHLF) [338,339]. In order to decrease complication rates variations on the technique have been developed, with decreased parenchymal transection in the first stage and longer inter-stages interval [340,341]. The ALPPS procedure induces a strong and swift growth of the FLR [249] but static volumetric criteria *per se* are probably not enough to guarantee adequate function [340,342]. The Kinetic Growth Rate (KGR), the percentage volumetric increase of the FLR per unit of time, has emerged as a sensitive marker of adequate liver function after portal vein embolization (PVE) or ALPPS [267,343].

The remarkably rapid regenerative response of ALPPS is characterized by a greatly enhanced proliferative index [251]. Since liver regeneration is a highly energy-dependent process [43], the ALPPS procedure probably relies on important metabolic adaptation of hepatocytes in order to sustain the immense energy requirements.

Mitochondria are the powerhouse of eukaryote cells, hosting the electron transport chain (ETC), the final common pathway for production of adenosine triphosphate (ATP). In the presence of oxygen, the ETC pumps protons from the mitochondrial matrix into the inter-membrane space, creating an electro-chemical gradient. The return of protons to the mitochondrial matrix is coupled to ATP synthesis by the F_1F_0 ATP-synthase. Moreover, mitochondria are key players in the cell death [33]. Mitochondrial dysfunction can cause necrotic cell death due to energetic failure; or

apoptotic cell death due to membrane permeabilization, release of cytochrome c into the cytoplasm and activation of Bax-mediated programmed cell death.

Mitochondrial function is an important factor in the liver's response to resection, providing the energy required for cell growth, division and maintenance of hepatocellular function [43,52,40]. Furthermore, intraoperative mitochondrial bioenergetics has been shown to correlate with posthepatectomy liver function [19]. The mitochondrial enzymatic machinery, including ETC enzymes such as cytochrome oxidase (COX) depends on the coordinated transcription of the nuclear and mitochondrial genome, which is regulated by several transcription factors, such as peroxisome proliferator-activated receptor-γ coactivator (PGC-1α) [34]. Mitochondrial membrane potential and respiration can be measured in biopsy samples and are excellent indicators of cellular energy homeostasis [270,271].

However, the metabolic and energetic adaptations of the FLR during two-stage hepatectomy, and particularly the ALPPS procedure, have not been investigated up to this day. Expanded knowledge on this field could pave the way for the discovery of novel markers of liver metabolic capacity, accurately staging tolerance to extended resection. And energetic conditioning of the liver parenchyma by pharmacologic modulation of mitochondrial function [20] could potentially decrease morbidity and mortality associated with the ALPPS procedure.

The main objectives pursued in this Chapter are:

1. To assess bioenergetics parameters in ALPPS patients and compare findings in stage 1 with matched controls undergoing minor atypical hepatectomies; and findings in stage 2 with matched patients undergoing major hepatectomy;
2. To correlate clinical and volumetric data in ALPPS patients with liver metabolic status;
3. To investigate the biological mechanisms associated with the regenerative response in the ALPPS procedure, in particular protein content and expression of genes associated with liver regeneration, energy metabolism and apoptosis.

II. Patients and Methods

Consecutive patients undergoing the ALPPS procedure and matched patients undergoing minor (miHp) and major hepatectomy (MaHp) in our department between September 2015 and September 2016 were included. Institutional ethics committee approved the study and informed consent was obtained from each patient.

Decision for resection was discussed in a multidisciplinary setting and patients were eligible for ALPPS if they had: good performance status; multiple bilobar metastases not amenable to one-stage parenchymal-sparing resection; adequate response to neoadjuvant chemotherapy; no extra-hepatic disease with the exception of resectable lung metastases; and a predicted FLR volume under 30% of the total standardized liver volume; or under 37.5% in patients with more than six cycles of chemotherapy, as reported by Narita et al [140].

A. Study population: ALPPS group

Study population (ALPPS group) consisted of five patients, three male and two female, with a median age of 59 years (interquartile range IQR 55–68). All patients had colorectal cancer liver metastases (CRLM), with a median number of eight nodules (IQR 6.5–12.5) and a median size of 44 mm (IQR 28.5–62.5) for the largest lesion. Preoperative chemotherapy was performed in all cases, with a median number of 14 cycles (IQR 6–15). Clinical variables of the ALPPS group are detailed in Table 4.1.

B. Operative procedures

Our department's operative technique for liver resection has been previously described [115,273] and we chose a partial ALPPS technique, according to a modification proposed by Linecker et al [341]. First-stage ALPPS (T1) consisted of atypical, parenchymal-sparing resections of liver metastases in the FLR, contralateral portal vein ligation (PVL) and limited (up to 50%) parenchymal transection along the

future line of division. Four cases underwent left FLR clearance and right PVL; and one patient underwent right posterior section FLR clearance and left PVL. A median number of three nodules (IQR 2-4) were resected in T1.

After a median time of 21 days (IQR 14–28), ALPPS patients underwent extended hepatectomy (stage 2, or T2): right extended to segment 4 in four cases; and left extended to segments 5 and 8 in one case.

Hepatic pedicle clamping (HPC), used only when deemed necessary (in an intermittent strategy of 15 minutes clamping with 5 minutes reperfusion), was performed for a shorter median time of 16 minutes (IQR 0–31) in T1 versus T2 (41 minutes IQR 19–45.5) (p=0.038). No transfusion was required in T1, while a median volume of packed red blood cells of 560 mL (IQR 0-1000) was transfused in T2.

C. Control groups: Minor and Major hepatectomy

Patients with CRLM scheduled to go one-stage parenchymal-conservative surgery served as matched controls for ALLPS T1 in the minor hepatectomy group (miHp group). And patients scheduled to undergo one-stage major hepatectomy (MaHp group) served as matched controls for ALPPS T2.

There were no significant differences in age, sex, biometric data or exposure to preoperative chemotherapy between the ALPPS and both control groups. The size of the largest nodule was larger in the ALPPS group but this was not statistically significant. As expected, ALLPS patients had a higher number of metastases, 8 (IQR 6.5–12.5), versus 3 (IQR 2–10) in the miHp group and 4 (IQR 2.5–5) in the MaHp group (p=0.009).

Operative technique was similar in ALPPS and controls, with the obvious exception of not performing PVL or parenchymal transection in miHp. There was also an expected difference in the volume of the FLR in the ALPPS group versus the MaHp, statistically significant before T1 (p=0.047) but barely significant before T2 (p=0.075).

Table 4.1 - Study population (n=5 patients undergoing ALPPS) and controls (n=10 patients undergoing minor and major hepatectomies)

Clinical and operative characteristics of cases (patients undergoing Associating Liver Partition and Portal Vein Ligation for Staged Hepatectomy - ALPPS) (n=5) and both control populations: patients undergoing minor (miHp n=5) and major hepatectomy (MaHp n=5). Continuous variables are presented as median and interquartile range.

	ALPPS n= 5	**Minor hepatectomy n= 5**	**Major hepatectomy n= 5**	**p**
Age (years)	59 (55 – 68)	59 (54 – 64)	58 (53 – 66)	0.671
Sex M/F	3 / 2	4/1	3 / 2	0.490
Diabetes mellitus Y/N	1 / 4	1/4	2 / 3	0.490
Body Mass Index ($kg.m^{-2}$)	24 (20.5 – 26.7)	24.2 (21.2 – 26)	27.6 (22.6 – 33.3)	0.251
Preoperative chemotherapy	5 (100%)	5 (100%)	5 (100%)	0.565
• **Irinotecan-based**	4	3	3	
• **Oxaliplatinum-based**	1	2	1	
• **Capecitabine**	0	0	1	
Number of cycles	14 (6 – 15)	9 (6 – 17)	9 (5.5 – 12)	0.292
Non-tumoral parenchyma				0.565
• **Normal**	4	4	3	
• **Mild sinusoidal dilation**	1	0	2	
• **Mild steatosis**	0	1	0	
Number of nodules	**8 (6.5 – 12.5)**	**3 (2 – 10)**	**4 (2.5 – 5)**	**0.009**
Size of largest nodule (mm)	44 (28.5 – 62.5)	30 (21 – 45)	30 (21 – 44)	0.295
Number of nodules resected				
• **T1**	3 (2 – 4)	3 (2 – 10)	-	0.690
• **T2**	5 (4 – 9)	-	4 (2.5 – 5)	0.310
Hepatic pedicle clamping time (minutes)				
• **T1**	16 (0 – 31)	25 (17 – 62)	-	0.463
• **T2**	41 (19 – 45.5)	-	44 (15.5 – 61.5)	0.673
Blood transfusion (mL)				
• **T1**	0	0	-	1
• **T2**	560 (0 – 1000)	-	480 (0 – 1100)	0.9
Future liver remnant volume (cm^3)				
• **Before ALPPS T1**	**330 (250 – 500)**	-	**721 (516 – 912)**	**0.047**
• **Before ALPPS T2**	450 (350 – 610)	-		0.075
Standardized Future Liver Remnant (%)				
• **Before ALPPS T1**	**26 (20 – 37)**	-	**49.8 (37.7 – 56.1)**	**0.05**
• **Before ALPPS T2**	36 (28 – 45)	-		0.076

Other variables, including number of resected lesions, duration of hepatic pedicle clamping (also used in an intermittent strategy) and intraoperative transfusion were all similar between ALPPS T1 and miHp group and ALPPS T2 and MaHp. Clinical, pathologic and operative variables are detailed in Table 4.1.

D. Volumetric and functional analysis

Volumetric analysis was performed using an imaging processing software (Osirix, Pixmeo, Geneva, Switzerland) on contrast-enhanced computed tomography (CECT) before and at a median time of 8 days (IQR 7-12) after T1. Standardized future liver remnant volume (sFLR) and Kinetic Growth Rate (KGR) were calculated according previous studies [267,343,344]. Growth rate (GR) was defined as the volume increase (in cm^3) divided by the number of days between T1 and the volumetric assessment with CT. The body surface area was calculated according to Mosteller [345].

Indocyanine green (ICG) clearance was assessed before T2, with a median retention rate of 5.2% (IQR 2.4-7.8) at 15 minutes.

Volumetric analysis for the MaHp group consisted of measurement of the volume of the FLR on the basis of the most recent preoperative CECT and calculation of the standardized future liver remnant volume (sFLR) with the same method as above. Because of the one-stage procedure no KGR was calculated in the MaHp group.

E. Collection of biopsies

Biopsies were performed in the FLR at two time-points: biopsy A, collected at the beginning of the procedure; and biopsy B, collected at the end of the procedure, immediately after the last period of clamping. One pair of biopsies was performed for each ALPPS patient during T1 and another pair during T2. Both miHp and MaHp patients had one pair of intraoperative biopsies. Thus, a total of 40 liver biopsies were collected from 15 patients.

Samples were immediately placed in 4° C preservation solution (sucrose 250 mM, ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid [EGTA] 0,5 mM, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid [HEPES] 10 mM, pH 7.4, bovine serum albumin [BSA 1%]) and immediately transported to the laboratory for mitochondrial fraction isolation and function tests. In ALPPS patients another sample was frozen at –80º C for assessment of gene expression and protein content.

1. Mitochondrial isolation, measurement of mitochondrial membrane potential and oxygen consumption

Mitochondria were isolated and membrane potential was estimated using an ion-selective electrode to measure the distribution of tetraphenylphosphonium (TPP^+) according to our previous work [19]. The measured parameters were: membrane potential (mV); depolarization (mV); lag phase (seconds); and repolarization (mV) (Figure 4.1.A). Values for baseline sample (biopsy A) and end of surgery sample (biopsy B) were obtained and the difference was calculated as the value in sample B minus the value in sample A.

Oxygen consumption of isolated mitochondria was polarographically determinated with a Clark oxygen electrode (Oxygraph, Hansatech Instruments Ltd, UK), also according to our previous work [19]. State 3 and Respiratory Control Ratio (RCR) were calculated according to Chance and Williams [277]. Initial and final values of RCR were obtained and the difference calculated, as above.

2. Gene expression

Ribonucleic acid (RNA) was isolated from flash-frozen liver samples from ALPPS patients with Aurum™ Total RNA Mini Kit (Bio-Rad, Hercules, CA. USA), according to the manufacturers' instructions. Total RNA yield was quantified with a Nanodrop instrument (Thermo Scientific, Waltham, MA. USA) and complementary DNA was produced using 1 µg of RNA with a iScript cDNA Synthesis Kit (Bio-Rad). Gene expression was evaluated by real-time Polymerase Chain Reaction (PCR) with

Sybr Green Supermix (Bio-Rad) in a C-1000 Touch CFX 96 Real-Time System equipment (Bio-Rad). Primers for the following genes were used: Signal Transducer and Activator of Transcription 3 (*STAT3*); Augmenter of Liver Regeneration (*ALR*); *Cyclin D1*; Hepatocyte Growth Factor (*HGF*); Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*PGC-1α*); Mitochondrial transcription factor A (*TFAM*); cytochrome oxidase subunit I (*COX1*); cytochrome oxidase subunit IV (*COX4*); small heterodimer partner (*SHP*); Sirtuin 1 (*SirT1*); and nicotinamide phosphoribosyltransferase (*Nampt*). The relative expression of each gene was normalized to the expression of I.

All primers were designed using the Primer3™ (Thermo Fisher) on-line software tool and validated using the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST). Primers were ordered from Thermo Fisher. Primer sequences are listed in Table 4.2. Data were presented as a ratio of content in biopsy B to basal expression in time-point A at each stage.

3. Measurement of protein content

Tissue homogenates were lysed in ice-cold RIPA lysis buffer (Sigma-Aldrich, MO. USA) supplemented with a cocktail of protease inhibitors. 50 µg of protein were loaded and electrophoresed on SDS-polyacrylamide gel and transferred to a polyvinylidene difluoride membrane. Membranes were blocked with 5% non-fat milk and incubated with anti-COX I (1:1000), anti-PGC-1α (1:100), anti-TFAM (1:2000), anti-NAMPT (1:1000), anti-SIRT1 (1:1000), anti-phospho-Sirtuin 3 (SIRT3) (1:500) or anti-BAX (1:1000), overnight at 4ºC. Immunodetection was performed with WesternDot 625 goat anti-rabbit or goat anti-mouse western blot kits. Membranes were imaged using a Versa Doc 3000 and the densitometry analysis was performed with the Image J software.

Table 4.2 - Primers used and corresponding nucleotide sequences for Real-Time Polymerase Chain Reaction (PCR) analysis of gene expression in ALPPS patients (n=5) on both stages

ALR/GFER: Augmenter of Liver Regeneration; *COX1*: cytochrome oxidase subunit I; *COX4*: cytochrome oxidase subunit IV; *HGF*: Hepatocyte Growth Factor; *Nampt*: nicotinamide phosphoribosyltransferase; *PGC-1α*: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; *SHP*: small heterodimer partner; *SirT1*: Sirtuin 1; *STAT3*: Signal Transducer and Activator of Transcription 3; *TFAM*: Mitochondrial transcription factor A

Gene	Upper	Lower
ALR/GFER	GGTGAGAGTGAGCCAGGAAC	TCCAAACCCGCCAAGAACTT
COX1	ACG TTG TAG CCC ACT TCC AC	CAT CGG GGT AGT CCG AGT AA
COX4	GCC ATG TTC TTC ATC GGT TT	TCA TGT CCA GCA TCC TCT TG
Cyclin D1	GGCGGAGGAGAACAAACAGA	TGTGAGGCGGTAGTAGGACA
HGF	CAATGCCTCTGGTTCCCCTT	CAGGGCTGACATTTGATGCC
Nampt	TCT TCA AGG ACC CAG TTG CT	TGA TGT GCT GCT TCC AGT TC
PGC-1α	CCT TGC AGC ACA AGA AAA CA	CTG CTT CGT CGT CAA AAA CA
SHP	GGA ATA TGC CTG CCT GAA AG	CTC CAA TGA TAG GGC GAA AG
SirT1	GCA GAT TAG TAG GCG GCT TG	TCT GGC ATG TCC CAC TAT CA
STAT3	TGGCACTTGTAATGGCGTCT	GTGTTCCCATACGCACAGGA
TFAM	CCG AGG TGG TTT TCA TCT GT	ACG CTG GGC AAT TCT TCT AA
18S	AAC GGC TAC CAC ATC CAA	TTT TCG TCA CTA CCT CCC

F. Postoperative serum biochemistry

Arterial lactate was measured every six hours in the first 24 hours. Standard biochemical determinations of International Normalized Ratio (INR), total bilirubin, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were performed every day until discharge. Arterial lactate clearance was calculated according to Wu et al [279]. In ALPPS patients glutamate dehydrogenase (GLDH) was assessed on postoperative days 1, 2 and 3 with a GLDH assay kit (Sigma-Aldrich) on Enzyme Linked Immunosorbent Assay.

G. Postoperative liver function and clinical course

Patients were extubated in the operating room and admitted to the surgical intensive care unit. Postoperative morbidity was defined and graded according to Dindo et al up to the 90th postoperative day [280]. Posthepatectomy liver failure (PHLF), was defined according to the "50-50" criteria [165] and graded by International Study Group of Liver Surgery (ISGLS) consensus definition [162]. Bile leakage was defined by the ISGLS definition [281].

H. Statistical analysis

Normality of distribution was confirmed with the Shapiro-Wilk test. All continuous variables were presented as mean ± standard deviation (SD) (if normally distributed) or median and interquartile range (IQR). Continuous variables with normal distribution were compared with two-tailed Student t test. Variables with non-normal distribution with the two-tailed Mann-Whitney U test. Categorical variables were compared with Chi-square test. Linear correlation between continuous variables was assessed with Pearson coefficient. Statistical analysis was performed using SPSS™ (version 21.0, SPSS inc., Chicago, IL. USA). Significance was considered when $p \leq 0.05$ and a statistical trend with $p \leq 0.1$.

III. Results

A. Clinical outcome and volumetric growth

There was no morbidity in the ALPPS group after T1. After T2, one patient (20%) developed grade A bile leakage (Dindo grade II) and one patient (20%) developed pleural effusion (Dindo grade II). One patients in the miHp group developed grade B biloma (20%) (Dindo grade IIIA). No major morbidity was observed in the

MaHp group, with one patient (20%) developing pleural effusion (Dindo grade II). No cases of PHLF or mortality were reported.

Median initial FLR volume before T1 was 330 cm^3 (IQR 250–500) and after a median of 8 days (IQR 7-12) increased to 450 cm^3 (IQR 350–610) (p=0.043). This corresponded to a pre-operative sFLR of 26% (IQR 20–37) and increased to 36% (IQR 28 – 45) before T2. The FLR to body weight ratio was 0.5% (IQR 0.4–0.8) before T1 and increased to 0.8% (IQR 0.6–0.9) after T1 (p=0.043). The volumetric growth in ALPPS corresponded to a KGR of 6.4%/day (IQR 2.9–8.2) and a GR of 8.1 cm^3/day (IQR 6.2–14.6). Volumetric data in the MaHp patient cohort are displayed in Table 4.1.

B. Liver bioenergetics compared: ALPPS T1, T2 and controls

In order to decrease bias, instead of comparing absolute values in bioenergetics parameters between ALPPS and controls, we compared the intraoperative variation between biopsy A and biopsy B. We found that bioenergetics suffered a slight but significant depression in ALPPS T1 versus miHp patients. In particular the lag phase, i.e., the time needed to repolarize the mitochondrial membrane potential after phosphorylation of all the ATP, increased 31.8±3.8 seconds in T1 versus an increase of only 11.2±16.9 in miHp (p=0.031) (Table 4.3).

Similarly, all measured bioenergetics parameters showed a slight but statistically significant intraoperative deterioration in ALPPS patients in stage 2, when compared with MaHp. In particular, the respiratory control ratio (RCR) experienced a larger drop in ALPPS (-1.0±0.5) versus MaHp, where there was an increase of 1.5±1.0 (p=0.001) (Table 4.3).

When comparing both stages of the ALPPS, we found a more pronounced intraoperative deterioration of lag phase in stage 1 than stage 2 (p<0.001) (Figure 4.1 A and B). Furthermore, there was a significant inter-stages improvement in mitochondrial respiration (p=0.039) (Figure 4.1.C).

Table 4.3 - Energy metabolism in the study population (n= 5 patients undergoing ALPPS) and controls (n= 10 patients undergoing minor and major hepatectomies)

Intraoperative variation in bioenergetics parameters in Associating Liver Partition and Portal Vein Ligation for Staged Hepatectomy (ALPPS n=5) stage 1 (T1) and minor hepatectomy controls (n=5); and in ALPPS stage 2 (T2) and major hepatectomy controls (n=5). Values are presented as mean ± standard deviation.

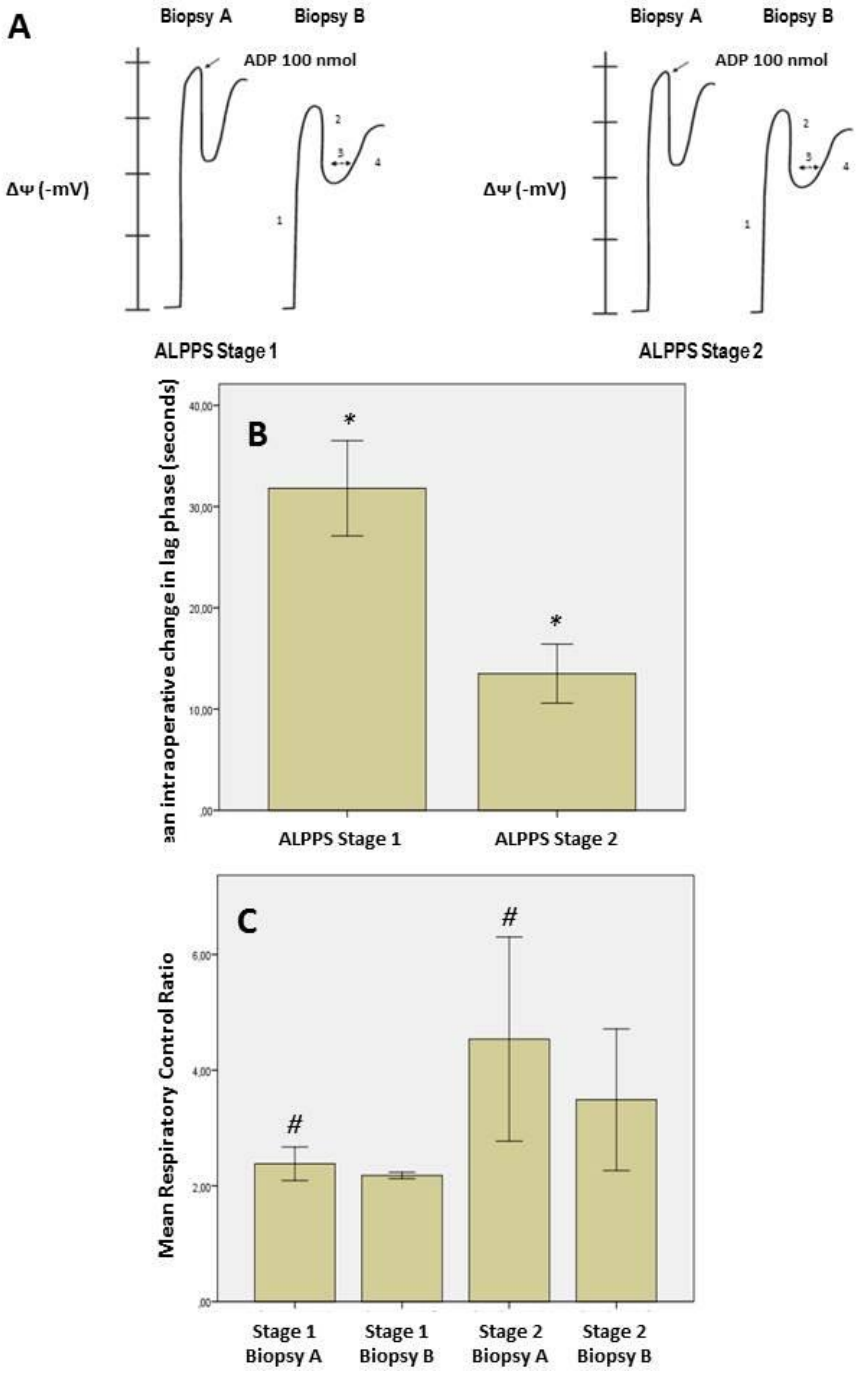
Intraoperative change in:	ALPPS T1	Minor hepatectomy	p	ALPPS T2	Major hepatectomy	p
Membrane potential (mV)	- 26.4 ± 4.5	- 8.9 ± 19.1	0.109	**- 30.6 ± 42.1**	**8.1 ± 9.9**	**0.025**
Lag phase (seconds)	**31.8 ± 3.8**	**11.2 ± 16.9**	**0.031**	**13.5 ± 2.3**	**- 8.7 ± 18.3**	**0.025**
Depolarization (mV)	- 13.2 ± 14.3	- 6.2 ± 8.6	0.372	**- 10.1 ± 6.8**	**0.67 ± 3.4**	**0.027**
Repolarization (mV)	**- 22.3 ± 6.6**	**- 6.4 ± 15.5**	**0.05**	**- 10.3 ± 0.5**	**6.4 ± 7.6**	**0.024**
Respiratory control ratio	- 0.2 ± 0.2	0.02 ± 1.2	1	**- 1.5 ± 0.5**	**1.5 ± 1.0**	**0.001**

Figure 4.1 - Bioenergetics parameters in both stages of the ALPPS procedure (n=5)

A. Representative graph of mitochondrial membrane potential and oxidative phosphorylation in one patient of the ALPPS group, as assessed in the tetraphenylphosphonium (TPP^+) ion-selective electrode. This graph demonstrates that several intraoperative parameters suffered a greater intraoperative worsening in stage 1 than in stage 2, namely: 1) decrease in membrane potential; 2) decrease in depolarization; 3) increased lag phase; 4) decreased repolarization

B. Intraoperative worsening of mitochondrial lag phase is more pronounced in stage 1 than in stage 2 of the ALPPS procedure (* $p < 0.001$)

C. Respiratory Control Ratio during both stages of the ALPPS procedure, in both sampled time-points, biopsy A at the beginning of the hepatectomy and biopsy B at the end. A significant improvement was observed from biopsy A of stage 1 to biopsy A of stage 2 (# $p = 0.039$)



C. Energy metabolism and postoperative liver remnant function and volume growth

Mitochondrial bioenergetics presented several strong and significant correlations with several markers of postoperative liver function in ALPPS patients. Post-T2 early lactate clearance strongly correlated with RCR (r=0.994; p=0.006) and membrane potential (r=0.969; p=0.031). Intraoperative change in mitochondrial membrane potential during T2 inversely correlated with postoperative day 1 ALT (r=-0.932; p=0.021) and AST (r=-0.969; p=0.006). Post-T2 fifth day INR correlated closely with membrane potential (r=-0.982; p=0.003) and repolarization (r=-0.957; p=0.011). On postoperative day 1 GLDH showed a very strong and significant correlation with arterial lactate (r=0.905; p=0.035).

Decreased time to repolarize the mitochondrial membrane potential in T1 significantly correlated with improved KGR (r=-0.945; p=0.015). And intraoperative increase in repolarization in T1 also demonstrated a very close linear correlation with improved FLR growth (r=0.989; p=0.001) (Figure 4.2). Furthermore, improved volumetrics also correlated with improved energetic status in T2, with the strongest correlation demonstrated of KGR with improved State 3 respiration (r=0.931; p=0.021) and GR with depolarization (r=0.919; p=0.027).

D. Gene expression and protein content in ALPPS

Liver samples in both stages of ALPPS were collected at the two time-points, beginning and end of surgery for analysis of gene expression and protein content.

RNA content of genes associated with liver regeneration (*STAT3*, *ALR*) ($p<0.05$), mitochondrial biogenesis (*PGC-1α*) (p=0.004) and energy metabolism (*COX1*) (p=0.003) significantly increased intraoperatively during ALPPS T2, but not during T1 (Figure 4.3). The expression of nicotinamide phosphoribosyltransferase (*Nampt*) (p=0.003) was also significantly increased during both stages of ALPPS (Figure 4.3) while the expression of *SHP* presented an intraoperative increase in T1 (p=0.034).

Furthermore, we also observed an increase in basal content of PGC-1α, COX1 and SIRT1 from T1 to T2 (p=0.006, p=0.002 and p=0.019, respectively) (Figure 4.4 A to D). There was a significant increase in the pro-apoptotic protein Bax content from time-point A to B in T1 (p=0.036), but not in T2; while the end of surgery (time-point B) content in Bax was lower in T2 than in T1 (p=0.015).

Figure 4.2 - Correlation between intraoperative change in mitochondrial energy parameters and future liver remnant volumetry in ALPPS patients (n=5)

A. Very strong negative correlation between intraoperative change in lag phase, ie the time needed to complete phosphorylate all ATP, and Kinetic Growth Rate (Pearson r= - 0.945; p = 0.015)
B. Very strong positive correlation between intraoperative change in repolarization and Growth Rate (Pearson r= 0.989; p = 0.001)

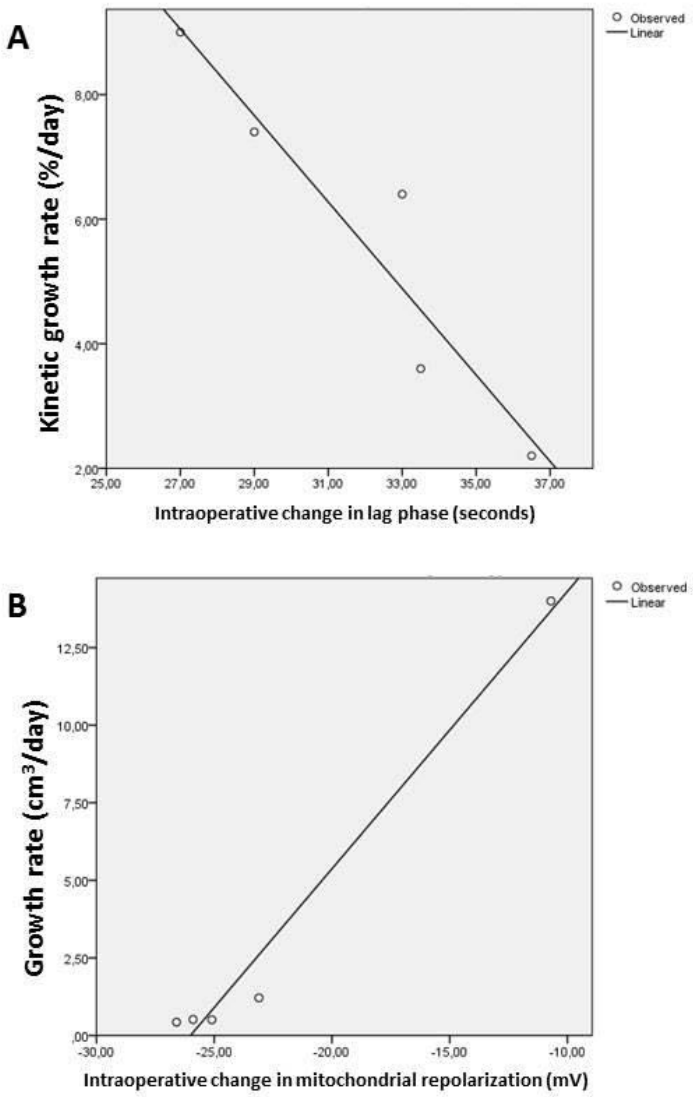


Figure 4.3 - Gene expression in biopsies obtained in the liver remnant in both stages of the ALPPS procedure (n=5)

Biopsies were collected at both time-points (time-point A at the beginning of the procedure, and time-point B at the end). Data are presented as a ratio of time-point B to basal expression in time-point A at each stage

A. Increase in expression of Signal Transducer and Activator of Transcription 3 (*STAT3*) at the end of stage 2 (* p = 0.036 versus sample A)

B. Increase in expression of Augmenter of Liver Regeneration (*ALR*) at the end of stage 2 (** p < 0.05 versus other samples)

C. Expression of short heterodimer partner (*SHP*) signficantly increased during stage 1 of ALPPS (*** p = 0.034)

D. Increase in expression of Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*PGC-1α*) at the end of stage 2 (**** p = 0.004 versus T1 and T2 sample A)

E. Increase in expression of cytochrome oxidase subunit 1 (*COX1*) at the end of stage 2 (# p = 0.003 versus other samples)

F. Increase in expression of nicotinamide phosphoribosyltransferase (*Nampt*) at the end of stage 1 (## p = 0.01 versus sample A) and at the end of stage 2 (### p = 0.003 versus sample A)

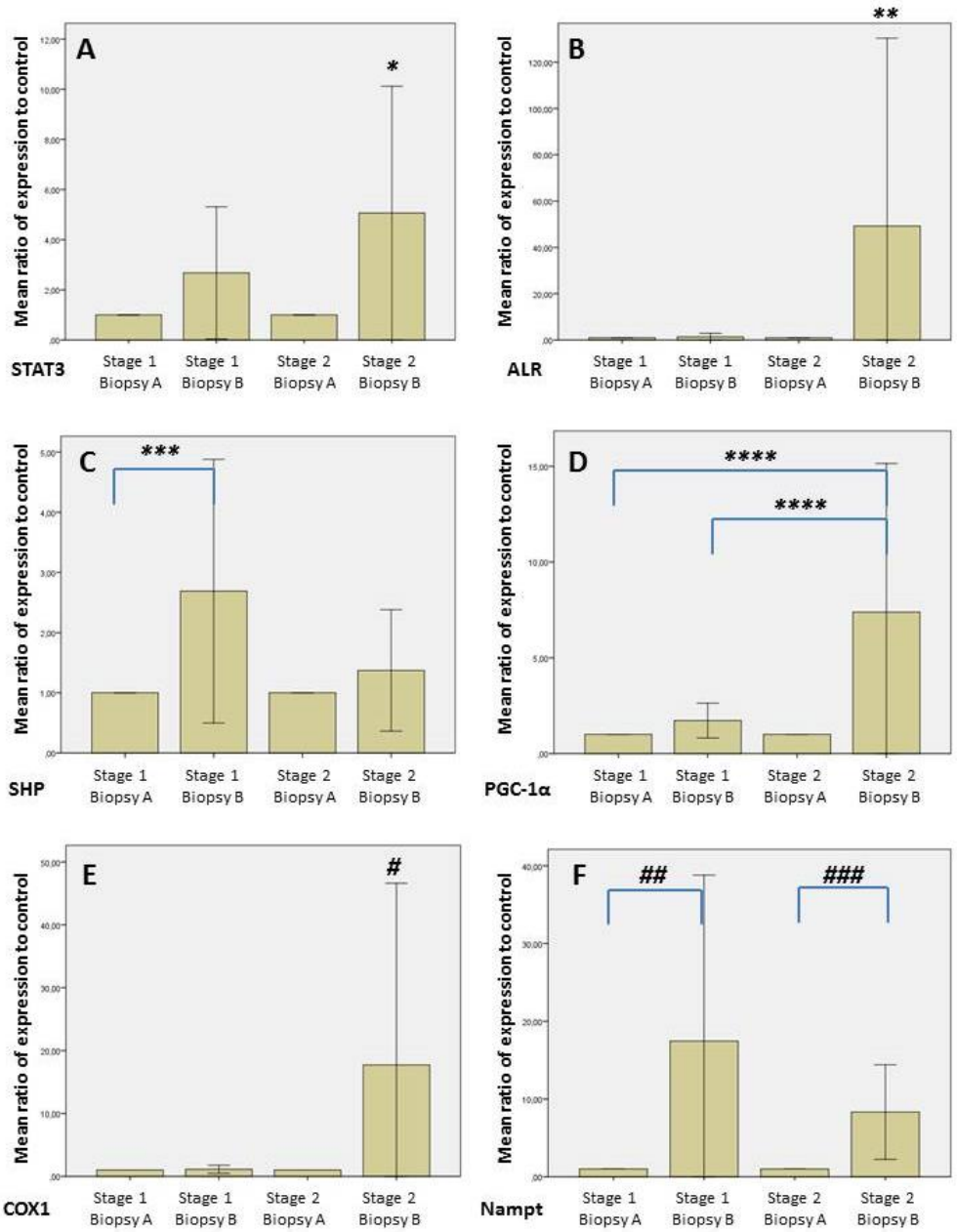
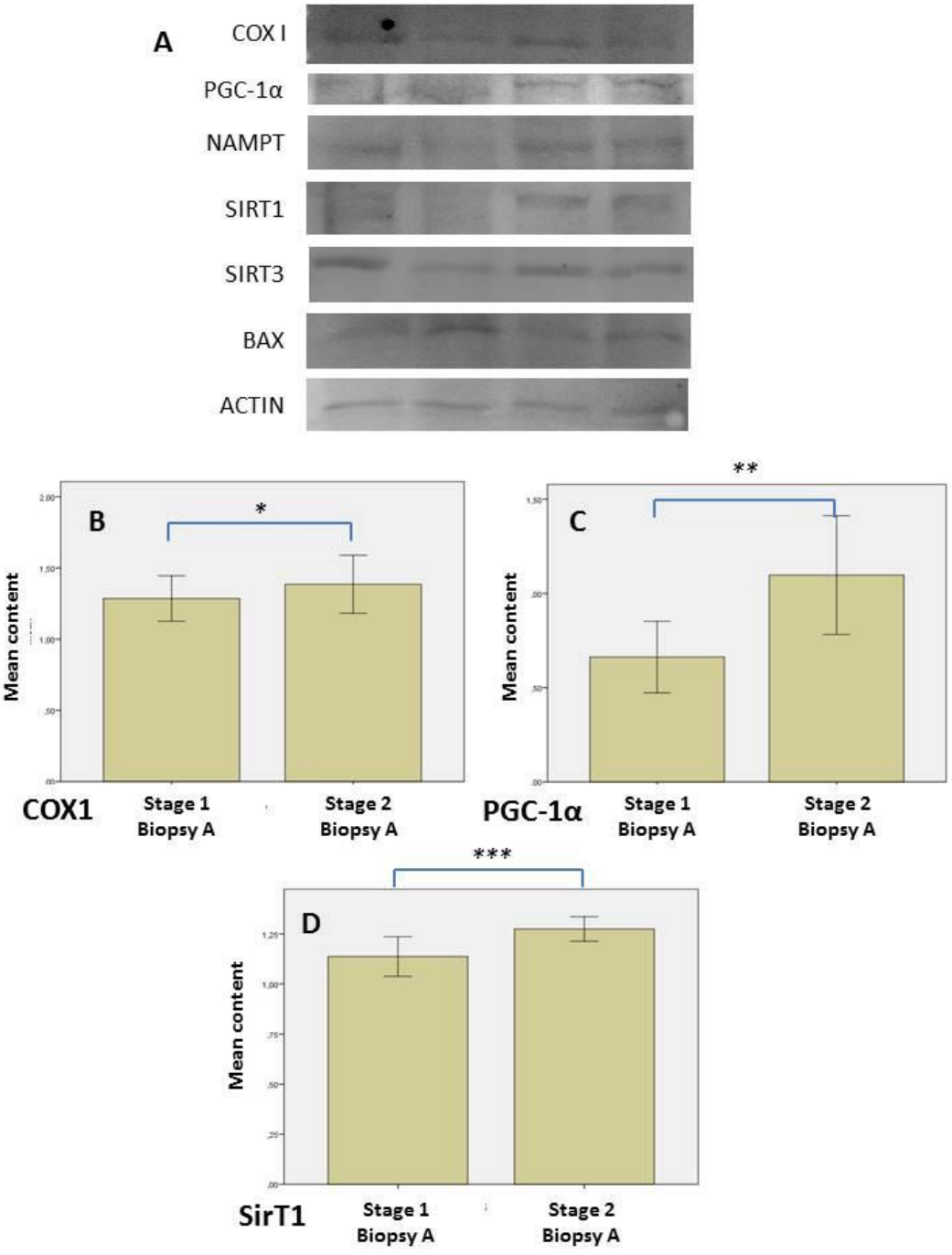


Figure 4.4 - Analysis of protein content (Western Blot) obtained in the liver remnant in both stages of the ALPPS procedure (n=5)

A. Representative Western blot analysis of protein content of several enzymes and transcription factors involved in energy metabolism, mitochondrial biogenesis and apoptosis
B. Slight but significant increase in cytochrome oxidase subunit I content in the liver remnant between stage 1 and stage 2 of ALPPS (* p = 0.006)
C. Significant increase in peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) content in the liver remnant between stage 1 and stage 2 of ALPPS (** p = 0.002)
D. Significant increase in Sirtuin 1 (SIRT1) content in the liver remnant between stage 1 and stage 2 of ALPPS (*** p = 0.019)



IV. Discussion

Liver regeneration, albeit a wondrous phenomenon, is not inexhaustible and some patients do not tolerate radical extended resections. Of the diverse techniques available in modern Hepatobiliary surgery, the ALPPS procedure probably relies on the extremes of the liver's regenerative capacity, allowing resection in otherwise inoperable patients [339]. This is clearly demonstrated by the successful completion of mono-segment ALPPS [346]. However, ALPPS is fraught with high morbidity and mortality, mostly due to PHLF [338].

Mitochondrial oxidative phosphorylation supplies the fuel for liver regeneration and liver energy status is known to correlate with posthepatectomy liver function and clinical outcome [19,41,43,52] (see also **Chapter I** - "Bioenergetics and Liver Regeneration: Review of the Role of Energy Status in the Pathophysiology of Posthepatectomy Liver Failure"). Experimental work has demonstrated that there is an increase in mitochondrial DNA and RNA in the remnant liver in the first hours after hepatectomy, as well as enhanced expression of several enzymes involved in energy metabolism, such as the ETC enzyme cytochrome oxidase (COX) [44,45]. This results in an overall increase of the liver's energy-producing capacity in the first two to four days after hepatectomy [46].

Mitochondrial quality and quantity in cells are influenced by the equilibrium of formation of new organelles (biogenesis) and its destruction (mitophagy) [34]. Several transcription factors are involved, including peroxisome proliferator-activated receptor-γ coactivator (PGC-1α), for the orchestrated transcription of both the nuclear and the 13-gene mitochondrial genome to produce new mitochondria. Experimental evidence has suggested that portal vein ligation (PVL) can improve mitochondrial function in the non-ligated lobes in a rat model, possibly mediated by an increase in mitochondrial biogenesis [245,246]. However, the adaptations occurring in human liver energy metabolism in two stage hepatectomies, including ALPPS are unknown.

We intended to explore the role of mitochondrial bioenergetics and biogenesis in the liver's regenerative response in ALPPS. By studying a cohort of five consecutive

patients undergoing the ALPPS procedure, we performed intraoperative liver biopsies in both stages, at two time-points, beginning (A) and end (B) of the procedure. With this we examined intraoperative changes in mitochondrial membrane potential and respiration in the remnant liver. The transmembrane potential developed by mitochondria upon addition of succinate is essential for energy production and is an extremely sensitive measure of metabolic capacity. And the Respiratory Control Ratio (RCR) reflects the capacity for substrate oxidation and ATP synthesis.

First we examined energy metabolism in both stages of ALPPS and compared them with matched controls. The control populations consisted of a cohort of patients undergoing multiple, minor parenchymal-sparing resections for stage 1; and a major hepatectomy for stage 2. Although the controls were adequately matched, unavoidable differences could present a bias in the interpretation of the results, as ALPPS is a more complex procedure: one-stage conservative resection does not include PVL or parenchymal division; and major hepatectomies presented an obviously larger FLR volume and were performed as a single stage procedure. So, in order to further reduce the individual variability in energetics parameters, in this comparison we only assessed the intraoperative change from biopsy A to biopsy B.

We found that mitochondrial function was hampered in both stages of ALPPS when compared to matched controls. Even after taking into account the aforementioned biases, this finding alerts to the possibility of mitochondrial dysfunction being a contributing factor in the higher risk of PHLF and mortality associated with ALPPS; and reinforces the caution surrounding its use [347].

However, we also found an extremely interesting improvement in mitochondrial respiration and function from T1 to T2, in spite of a slightly longer duration of HPC in the latter. Furthermore, we also describe a larger increase in the mitochondrial-dependent apoptotic protein Bax in T1 versus T2. These findings suggest that important metabolic adaptations occur in the FLR, promoting improved bioenergetics performance and decreased apoptosis. To our knowledge this has never been documented before.

Another interesting result was the correlation of mitochondrial energetics with posthepatectomy liver function, which seems to confirm previous work from our group

[19]. Although we experienced no cases of PHLF in this series, we found that mitochondrial membrane potential in T2 closely correlated in a negative way with two key markers of posthepatectomy liver function, peak arterial lactate and postoperative day 5 INR [11,171]. Furthermore GLDH, a mitochondrial matrix urea cycle enzyme and a very sensitive marker of liver necrosis and mitochondrial dysfunction [86,187], was tightly correlated with arterial lactate. And postoperative aminotransferases were also correlated with mitochondrial membrane potential. These results suggest that patients with better intraoperative energy metabolism presented improved postoperative liver function and decreased hepatocellular necrosis.

Also extremely interesting was the finding of a very close association of improved cellular energetics with enhanced volume growth of the FLR. In fact, we demonstrated that improved mitochondrial potential in T1 correlated with improved volume gain. Although the small size of the series should lead to caution in the interpretation of these results, this has, to our knowledge, also never been reported previously in the scientific literature. And were the main reason to further expand our study on this subject with detailed analysis of the expression of genes associated with liver regeneration, mitochondrial biogenesis and energy metabolism.

The regenerative response of ALPPS and other two-stage hepatectomies is linked to portal vein ligation (PVL). Like portal vein embolization (PVE), PVL shunts portal blood away from the tumour-bearing parenchyma and into the FLR, causing atrophy and apoptosis in the embolized hemiliver; and hypertrophy and increased proliferation in the non-embolized hemiliver [348]. This is probably due to the exposure of the FLR to higher concentrations of hepatotrophic factors from the gut. Possible implicated molecules are bile acids and enterohormones, such as glucagon-like peptide 1 (GLP-1) [349,350]. Our finding of increased expression of SHP in stage 1 is suggestive of the role of bile acids, as SHP is a pleiotropic transcription factor just downstream of the Farnesoid X Receptor (FXR), which is the main bile acid-regulated nuclear receptor involved in liver regeneration [351].

However, ALPPS differs from other two-stage hepatectomies because PVL is supplemented by the in situ splitting (total or partial) of the liver, separating the FLR from the tumour containing contralateral lobe. This parenchymal division causes a

surgical disconnection of left to right cross-portal shunts and undoubtedly contributes to the enhanced regenerative response [352]. But the augmented liver growth is also possibly linked with the increased systemic inflammation caused by the parenchymal partition. Although increased expression of Interleukin 6 (IL-6) was not found in a rabbit model of ALPPS [353], extra-hepatic injury with PVL is associated with similar volume gain in a mouse model of ALPPS and this is possibly mediated by the IL-6-STAT3 pathway [250]. Cytokines are known primers of liver regeneration and the STAT3 pathway can significantly improve mitochondrial function [16,354]. Moreover, mitochondrial respiration is improved in animal models of moderate but not severe abdominal sepsis [355]. Our results are in support of these observations, as we describe a significantly higher liver remnant expression of STAT3 at the end of stage 2.

Another interesting finding was the increased expression of Augmenter of Liver Regeneration (ALR), a potent hepatotrophic factor with important regulatory function in cellular respiration. ALR, also known as Hepatic Stimulator Substance or Hepatopoietin, is one of the strongest hepatic cell mitogens and it also modulates oxidative phosphorylation, mitochondrial biogenesis and ATP synthesis [200]. In fact, ALR's importance in energy metabolism seems to be dependent upon its effect on mitochondrial biogenesis, upstream of other two co-factors, TFAM and PGC-1α [21].

Also testifying the role of energy metabolism in liver regeneration was the fact that Nicotinamide phosphoribosyltransferase (Nampt), a key enzyme in the synthesis of nicotinamide adenine dinucleotide (NAD, an intermediate in the electron transfer between the citric acid cycle and the ETC) was highly expressed in the end biopsy of both stages of ALPPS. Nampt has recently been demonstrated to be involved in the energetic adaptations in experimental liver resection [22].

Finally, we think we have confirmed the importance of mitochondrial biogenesis and energy metabolism in Human liver regeneration. This is supported by the enhanced gene expression and increased protein content in T2 of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), a pivotal regulator of mitochondrial biogenesis; and of cytochrome oxidase subunit I (COX1), a component of complex IV of the ETC which is coded by the 13-gene mitochondrial genome. There

was also an increase in SIRT1 content, a metabolic sensor that acts directly on PGC-1α [36].

In conclusion, this work clearly demonstrates, for the first time in the scientific literature, the importance of adaptations in energy metabolism in the regenerative response of the liver in two stage hepatectomies. Of striking relevance are the inter-stages improvement in energy status and the correlation with volumetric growth. A definite role for mitochondrial biogenesis in this process is confirmed. In fact, the first stage of ALPPS seems to not only induce liver regeneration but also prompts energetic conditioning of the FLR, inasmuch as exercise does for striated muscle [36]. In the clinical setting liver regeneration is very likely tightly paralleled by an efficient mitochondrial response, in order to provide the ATP to fuel the extremely high metabolic requirements of swift cell replication.

The clinical implications of this study are twofold. First, bioenergetic status could be used as a liver functional reserve test, by staging parenchymal capacity to undergo extended resection. In fact, assessment of fitness to undergo stage 2 of ALPPS is increasingly performed with functional tests such as hepatobiliary scintigraphy, rather than volumetric-only tests [342]. The metabolism, conjugation and biliary excretion of iminodiacetic acid (and other xenobiotics such as indocyanine green), like most liver functions, is an endergonic, i.e. energy-requiring or ATP consuming reaction. Considering that liver regeneration is just another, although seldom required, liver function, we postulate that the assessment of energy status of liver cells could indicate liver pre-resection reserve [269]. This could assist in the surgical decision-making process: second stage of ALPPS could be performed earlier in patients with improved cellular bioenergetics; and later, or not at all, in patients with underlying mitochondrial dysfunction. However, as there were no cases of PHLF in our series we cannot speculate on the accuracy of this method and further studies are needed to clarify this issue.

Secondly, these findings open the way to the possibility of using mitochondrial-targeted pharmacological therapies to improve cellular bioenergetics in clinical liver surgery, enhancing liver regeneration and decreasing the incidence of PHLF. Several drugs are currently under evaluation in the preclinical setting [20,204,206,207] and

could potentially translate into the clinical arena, reducing the morbidity and mortality of liver surgery and hopefully expanding the current limits of hepatectomy.

Concluding remarks

Liver resection offers improved survival to patients with both primary and secondary liver malignancies. While doing so, modern Hepatobiliary Surgery constantly challenges the liver's unique ability to regenerate. In fact, technical constraints to resection are usually only imposed by the quality and volume of the predicted future liver remnant. With adequate preoperative planning and preparation, thorough selection of patients, meticulous surgical technique, dedicated intraoperative management and close postoperative care, the rule is that the majority of patients undergo resection of a significant fraction of parenchyma with an unimpeded recovery of mass and function of the liver remnant. Notwithstanding, on occasion the liver's capacity to regenerate is pushed beyond its limits, resulting in a syndrome of decreased excretory and synthetic functions known as Posthepatectomy Liver Failure (PHLF). Although often self-limited, the consequences of PHLF can at times be more portentous, with impaired renal function, increased risk of sepsis and death. Furthermore, evidence has emerged for a deleterious effect on long-term outcome in patients surviving PHLF.

Multiple experimental and some notable clinical evidence has proved that liver regeneration is a highly energy-dependent sequence of events. As was thoroughly reviewed in this Doctoral Thesis, the energy source for liver regeneration is mitochondrial oxidative phosphorylation, the highly efficient oxygen-dependent metabolic chain that fuels most processes in higher heterotrophic organisms. However, the link of mitochondrial function and postoperative outcome had not been clearly documented up to this day. In particular, the parallel of mitochondrial energy status and posthepatectomy liver dysfunction and morbidity remained unproven.

In this Thesis we sought to explore this relationship, and succeed in doing so. In one of the original clinical studies herein displayed, intraoperative decrease of mitochondrial membrane potential was confirmed for the first time as an independent factor in the development of postoperative liver-specific morbidity, with posthepatectomy liver function closely correlating with several bioenergetics parameters; for example, a longer lag phase, reflecting a prolonged time to phosphorylate adenosine diphosphate, was both highly sensitive and specific of the development of PHLF.

Furthermore, Hepatic Pedicle Clamping (HPC), an almost universal manoeuvre in the technical arsenal of liver surgeons, was demonstrated to have a distinct effect on mitochondrial function. This is an extremely interesting finding as it has long been known that longer duration of HPC, although causing ischemia-reperfusion injury is not linearly associated with worse postoperative outcome. Thus, mitochondrial susceptibility to HPC could in fact be a missing link in this equation and it was possible, in our work, to demonstrated this relationship; both in clinical and experimental studies.

Chemotherapy offers a major contribution to the management of patients with colorectal cancer liver metastases (CRLM), the most common indication for liver resection in developed nations. By reducing the size of liver nodules, resection can be offered to a significant proportion of patients initially deemed inoperable. However, one of the unwanted consequences of chemotherapy is the development of liver injury in three distinct patterns: steatosis, steatohepatitis and sinusoidal obstruction syndrome (SOS). Although mitochondrial dysfunction is involved in the pathophysiology of many liver diseases, its role in the pathogenesis of chemotherapy-associated liver injury (CALI) was still unknown. Furthermore, the exact clinical consequences of CALI are still under intense scrutiny, as a recent meta-analysis failed to prove the current notion of worse postoperative outcome in SOS.

This led to the pursuit of two studies. First, a clinical and pathological review of patients undergoing liver resection for CRLM was performed, confirming SOS as an independent factor in overall and liver-specific morbidity. However, the association of more severe forms of SOS with PHLF or mortality was not confirmed on multivariate analysis. Interestingly, steatosis and steatohepatitis did not hamper postoperative liver function or clinical outcome, challenging a deeply embedded dogma in the surgical community. The second study attempted to explore the possible involvement of mitochondrial dysfunction in the pathogenesis of SOS. The experimental study designed to answer these questions failed to replicate the characteristic histologic injury of a previously published model. Nonetheless, mitochondrial dysfunction was for the first time demonstrated to occur in an experimental model of chemotherapy-induced hepatotoxicity.

Although indications are constantly increasing, the technical limits of liver resection are presently being reached. But an incidentally discovered development recently expanded these boundaries, up to the point where, unthinkable a few years ago, the future liver remnant would consist of a single Couinaud segment. The Associating Liver Partition and Portal Vein Ligation for Staged Hepatectomy (ALLPS) procedure, an exciting but nonetheless risky procedure, probably dwells on the extremes of the liver's regenerative capacity. However, the precise adaptations in remnant liver bioenergetics occurring along with the profound inter-stages changes of ALPPS had never been documented. Again, this Thesis interrogated on this issue and several conclusions were drawn. First, mitochondrial function is worse in ALPPS than in other hepatectomies, a finding that is in accordance with the increased postoperative morbidity and mortality associated with this technique; and that reinforces the caution surrounding its use [347]. Moreover, the extremely interesting correlation of liver remnant mitochondrial membrane potential in the first stage with the inter-stages volume growth rate had never before described in the scientific literature. And finally, the inter-stages improvement in mitochondrial function suggested that important adaptations were taking place in the future liver remnant. This was confirmed by the paralleled expression profile of several key genes in liver regeneration, mitochondrial biogenesis and energy metabolism, an exciting first ever description in the scientific literature.

In conclusion, the research questions drawn at the start of this project were answered. However, several unresolved issues remain to be addressed in future research and will now be summarily catalogued.

In the first place, the exact mechanism of energetic dysfunction in major hepatectomies deserves further scrutiny. An attractive theory is the portal vein hyperflow and resultant increased shear stress. Although it does not explain the deterioration in liver function that, albeit rare can occur in minor hepatectomies, this theory elegantly fits the current knowledge on pathogenesis of PHLF in major and extended hepatectomies. However, shear stress alone might not justify the pathogenesis of the small-for-size syndrome. In fact, decreased arterial flow mediated by the Hepatic Artery Buffer Response could be the most relevant factor. The resulting parenchymal hypoxia would compromise mitochondrial energy production and hence clinical

outcome. In order to verify this theory, a large animal experimental model of extended hepatectomy would be needed in order to correlate liver macrovascular changes (including arterial and portal flow, pressure and shear stress), parenchymal oxygenation and cellular energy metabolism. Furthermore, the effects of intraoperative modulation of portal vein and hepatic artery flow on energy metabolism, liver function and outcome would be more readily assessed.

Another key point needing clarification is the mechanistic effect of oxaliplatin on mitochondrial function. In order to examine this question, a complete dissection of the molecular events taking place would probably require at least one study on hepatocyte culture. However, since SOS is also a sinusoidal endothelial cell disease, a three-dimensional co-culture comprising distinct liver cell populations would be a more appropriate research model. Hopefully, this would provide a significant contribution to the yet elusive pathophysiology of chemotherapy-induced SOS.

And as the evidence for mitochondrial dysfunction in the pathogenesis of PHLF builds up, it is unavoidable to consider future clinical applications. In this regard, three distinct pathways have their start at the “bench” and could lead to the “bedside”.

First, perioperative and early postoperative non-invasive assessment of mitochondrial function could assist in the timely recognition of PHLF. While mitochondrial function testing in liver biopsy samples is undoubtedly the gold standard, it is invasive and cumbersome. Thus, other promising modalities will surely find their way, notably serum glutamate dehydrogenase and microdialysis. Further research on these methods is warranted and could ensure accurate and early detection of posthepatectomy liver dysfunction.

Most Hepatobiliary surgeons would agree that estimation of hepatic preoperative reserve is one of the most important steps in the prehepatectomy decision-making process. And this leads to the second translation point of the findings herein included. Composite liver function tests alongside with remnant liver volume measurement have for long been the gold standard [269]. Although useful, these tests do not account for regional asymmetries in liver function. But nowadays other methods are allowing assessment of segmental liver function and are proving to be invaluable. Since most liver functions require energy, an estimation of liver energy capacity could prove to be

an excellent liver reserve test. However, the best way to achieve this goal is not clear at the present moment. As was demonstrated in this Thesis, direct assessment of liver mitochondrial function is possible in liver remnant biopsy samples collected during the first stage of a two-stage hepatectomy, such as the ALPPS procedure. However, one-stage resections would require a preoperative liver percutaneous liver biopsy to assess mitochondrial function, which although feasible is an invasive and risky procedure [183]. Ideally, appraisal of liver energy status should be performed with a non-invasive method. With the ability to measure the ATP/Pi ratio, ^{31}Phosphorus Magnetic Resonance Spectroscopy could aid in this quest, with the added advantage of assessing segmental liver function with gadoxetic acid enhanced study, and also measuring future liver remnant volume, as an all-in-one exam [128,268,356].

The third translation point is the extremely appealing potential for improving the liver's regenerative capacity with mitochondrial-based therapies. Although innovative techniques such as ALPPS or simultaneous portal and hepatic vein embolization are pushing the current limits of technical resectability beyond expectations [357,358], these limits will be ultimately met nonetheless. Considering energy status as one of the limiting factors in liver regeneration, energetic conditioning could potentially help transcend the boundaries of liver resection. Several candidate therapies were reviewed earlier and a few demonstrate enormous potential, such as: enhancing mitochondrial biogenesis with new drugs such as berberine; decreasing permeability transition with novel cyclophilin inhibitors; improving parenchymal oxygenation with hyperbaric oxygen and/or portal flow modulation; and upgrading mitochondrial pool with improved mitophagy. Some the aforementioned approaches could eventually find their way into the clinical arena and hopefully decrease morbidity and mortality of liver resection.

This Doctoral Thesis hopes to have provided a modest yet significant contribution to the understanding of Posthepatectomy Liver Failure. Mitochondria, the living relic of an early symbiotic relationship in Earth's life, relentlessly provide liver cells with the energy for regeneration. Their malfunction can severely impair clinical results after hepatectomy, either through depression of cell function, mitochondrial-induced programmed cell death or outright energetic failure and necrosis. And while playing a distinct yet undefined role in the complex changes that the liver suffers after

chemotherapy, mitochondrial function is confirmed as a key vector in the liver's regenerative response in two-stage hepatectomies.

Meanwhile, we feel that it is incumbent upon Surgeons in Academic Centres to foster basic and clinical research on this subject. Not only will this allow a deeper understanding of the pathophysiology of PHLF, but hopefully aid in its prevention and early detection. And with the development of energetic-conditioning strategies in the near future, we will conceivably provide hope for patients nowadays deemed unfit for surgery due to a predicted insufficient liver remnant. One dares to dream that those days are nearer than imagined.

Finally, while the mechanisms underlying liver regeneration have been the focus of intensive research in clinical and basic sciences, the most upstream primer of liver regeneration has yet to be identified. Metabolic stimuli, namely a change in whole organism energy status, could in fact be the igniter of hepatocyte proliferation [24,26]. Since liver resection is not a usual environmental stressor, the liver must have evolved the capacity for regeneration because of the constant exposure to toxic, metabolic and infectious aggressors carried by the portal blood flow. Given the central role of the liver in glucose and lipid metabolism, it is conceivable that liver regeneration has thus evolved as an adaptive response to two divergent phenomena: first, liver injury can significantly decrease liver mass; and second, fluctuations in whole-body energy supply and demand require enormous plasticity of the liver parenchyma. A hepatostat, i.e. a sensor of hepatocyte mass to whole body metabolic needs fits nicely with the theory of liver mass regulation by metabolic rather than hemodynamic stimuli [23,359–361].

Could it be that the hepatostat is none other than an elegantly placed intracellular energy sensing system? Could the stimulus for liver regeneration start with an altered cellular energy state, and thus the hepatostat would in reality be an "energostat"? An interesting parallel emerges: as much as the Liver must recuperate lost mass to maintain whole-body energy status, so Mitochondria should increase energy supply to fuel hepatocyte replication and function. Perhaps Mitochondria's role in Liver Regeneration is not simply as the provider of energy. Perhaps by sensing the hepatocyte's metabolic overload, they also play a role in the key events initiating Liver Regeneration. Surely

this question would fuel the scientific curiosity to mandate further and more exciting research. Energy will hopefully be applied in this endeavour.

Coimbra, March 2017

References

1. Bismuth H. Surgical anatomy and anatomical surgery of the liver. World J Surg. 1982 Jan;6(1):3–9.

2. Adam R, Delvart V, Pascal G, Valeanu A, Castaing D, Azoulay D, et al. Rescue surgery for unresectable colorectal liver metastases downstaged by chemotherapy: a model to predict long-term survival. Ann Surg. 2004;240(4):644-657-658.

3. Narita M, Oussoultzoglou E, Jaeck D, Fuchschuber P, Rosso E, Pessaux P, et al. Two-stage hepatectomy for multiple bilobar colorectal liver metastases. Br J Surg. 2011 Oct;98(10):1463–75.

4. Torzilli G, Belghiti J, Capussotti L, Vauthey J, Choti MA, Santibanes E De, et al. A Snapshot of the Effective Indications and Results of Surgery for Hepatocellular Carcinoma in Tertiary Referral Centers: Is It Adherent to the EASL / AASLD Recommendations? Ann Surg. 2013;257(5):929–37.

5. Nagino M, Ebata T, Yokoyama Y, Igami T, Sugawara G, Takahashi Y, et al. Evolution of Surgical Treatment for Perihilar Cholangiocarcinoma. Ann Surg. 2013;258(1):129–40.

6. Adam R, Chiche L, Aloia T, Elias D, Salmon R, Rivoire M, et al. Hepatic resection for noncolorectal nonendocrine liver metastases: analysis of 1,452 patients and development of a prognostic model. Ann Surg. 2006 Oct;244(4):524–35.

7. Clavien P-A, Petrowsky H, DeOliveira ML, Graf R. Strategies for safer liver surgery and partial liver transplantation. N Engl J Med. 2007 Apr 12;356(15):1545–59.

8. Golse N, Bucur PO, Adam R, Castaing D, Sa Cunha A, Vibert E. New paradigms in post-hepatectomy liver failure. J Gastrointest Surg. 2013 Mar;17(3):593–605.

9. Hammond JS, Guha IN, Beckingham IJ, Lobo DN. Prediction, prevention and management of postresection liver. Br J Surg. 2011;98(9):1188–200.

10. Qadan M, Garden OJ, Corvera CU, Visser BC. Management of Postoperative Hepatic Failure. J Am Coll Surg. 2016 Feb;222(2):195–208.

11. Reissfelder C, Rahbari NN, Koch M, Kofler B, Sutedja N, Elbers H, et al. Postoperative course and clinical significance of biochemical blood tests following hepatic resection. Br J Surg. 2011 Jun;98(6):836–44.

12. Nakamura N, Hatano E, Iguchi K, Seo S, Taura K, Uemoto S. Posthepatectomy Liver Failure Affects Long-Term Function After Resection for Hepatocellular Carcinoma. World J Surg. 2015 Nov 20;40(4):929–36.

13. Vibert E, Pittau G, Gelli M. Actual incidence and long-term consequences of posthepatectomy liver failure after hepatectomy for colorectal liver metastases. Surgery. 2014;155(1):94–105.

14. Capussotti L, Vigano L, Giuliante F, Ferrero A, Giovannini I, Nuzzo G. Liver dysfunction and sepsis determine operative mortality after liver resection. Br J

Surg. 2009;96(1):88–94.

15. Minuk GY. Hepatic regeneration: If it ain't broke, don't fix it. Can J Gastroenterol. 2003;17(7):418–24.

16. Fausto N, Campbell JS, Riehle KJ. Liver regeneration. Hepatology. 2006 Feb;43(2 Suppl 1):S45-53.

17. Michalopoulos GK. Liver regeneration: alternative epithelial pathways. Int J Biochem Cell Biol. Elsevier Ltd; 2011 Feb;43(2):173–9.

18. Tralhão JG, Abrantes AM, Hoti E, Oliveiros B, Cardoso D, Faitot F, et al. Hepatectomy and liver regeneration: from experimental research to clinical application. ANZ J Surg. 2013 May 8;84(9):665–71.

19. Alexandrino H, Varela AT, Teodoro JS, Martins MA, Rolo AP, Tralhão JG, et al. Mitochondrial bioenergetics and posthepatectomy liver dysfunction. Eur J Clin Invest. 2016 Jul;46(7):627–35.

20. Rehman H1, Sun J, Shi Y, Ramshesh VK, Liu Q, Currin RT, Lemasters JJ ZZ. NIM811 prevents mitochondrial dysfunction, attenuates liver injury, and stimulates liver regeneration after massive hepatectomy. Transplantation. 2012;91(4):406–12.

21. Han L-H, Dong L-Y, Yu H, Sun G-Y, Wu Y, Gao J, et al. Deceleration of liver regeneration by knockdown of augmenter of liver regeneration gene is associated with impairment of mitochondrial DNA synthesis in mice. Am J Physiol Gastrointest Liver Physiol. 2015 May 14;309(2):112–22.

22. Mukherjee S, Chellappa K, Moffitt A, Ndungu J, Dellinger RW, Davis JG, et al. Nicotinamide adenine dinucleotide biosynthesis promotes liver regeneration. Hepatology. 2016 Nov 3;65(2):616–63.

23. Huang J, Rudnick DA. Elucidating the Metabolic Regulation of Liver Regeneration. Am J Pathol. American Society for Investigative Pathology; 2014;184(2):309–21.

24. Crumm S, Cofan M, Juskeviciute E, Hoek JB. Adenine nucleotide changes in the remnant liver: An early signal for regeneration after partial hepatectomy. Hepatology. 2008 Sep;48(3):898–908.

25. Guerra MT, Fonseca EA, Melo FM, Andrade VA, Aguiar CJ, Andrade LM, et al. Mitochondrial calcium regulates rat liver regeneration through the modulation of apoptosis. Hepatology. 2011;54(1):296–306.

26. Ngala Kenda JF, de Hemptinne B, Lambotte L. Role of metabolic overload in the initiation of DNA synthesis following partial hepatectomy in the rat. Eur Surg Res Eur Chir Forschung Rech Chir Eur. 1984;16(5):294–302.

27. Yin F, Cadenas E. Mitochondria: the cellular hub of the dynamic coordinated network. Antioxid Redox Signal. 2015 Apr 20;22(12):961–4.

28. Lemasters JJ, Theruvath TP, Zhong Z, Nieminen A-L. Mitochondrial calcium

and the permeability transition in cell death. Biochim Biophys Acta. Elsevier B.V.; 2009 Nov;1787(11):1395–401.

29. Koolman J, Roehm K-H. Koolman, Color Atlas of Biochemistry. Koolman J, Roehm K-H, editors. Color Atlas of Biochemistry. Thieme; 2005. 215 p.

30. Stillway W. Bioenergetics and Oxidative Metabolism. In: Dominiczak M, Baynes J, editors. Medical Biochemistry. Mosby, Inc.; 1999. p. 83–94.

31. Rolo AP, Teodoro JS, Palmeira CM. Role of oxidative stress in the pathogenesis of nonalcoholic steatohepatitis. Free Radic Biol Med. 2012 Jan 1;52(1):59–69.

32. Paradies G, Paradies V, Ruggiero FM, Petrosillo G. Oxidative stress, cardiolipin and mitochondrial dysfunction in nonalcoholic fatty liver disease. World J Gastroenterol. 2014 Oct 21;20(39):14205–18.

33. Luedde T, Kaplowitz N, Schwabe RF. Cell death and cell death responses in liver disease: mechanisms and clinical relevance. Gastroenterology. 2014 Oct;147(4):765–783.e4.

34. Stotland A, Gottlieb RA. Mitochondrial quality control: Easy come, easy go. Biochim Biophys Acta. 2015 Jan 14;1853(10 Pt B):2802–11.

35. Wenz T. Regulation of mitochondrial biogenesis and PGC-1α under cellular stress. Mitochondrion. 2013 Mar;13(2):134–42.

36. Duarte F V, Amorim JA, Palmeira CM, Rolo AP. Regulation of Mitochondrial Function and its Impact in Metabolic Stress. Curr Med Chem. 2015;22(20):2468–79.

37. Madrigal-Matute J, Cuervo AM. Regulation of Liver Metabolism by Autophagy. Gastroenterology. 2016 Feb;150(2):328–39.

38. Lee S, Kim J-S. Mitophagy: therapeutic potentials for liver disease and beyond. Toxicol Res. 2014 Dec 31;30(4):243–50.

39. Nakatani T, Yasuda K, Ozawa K, Kawashima S, Tobe T. Effects of (+)-octanoylcarnitine on deoxyribonucleic acid synthesis in regenerating rabbit liver. Clin Sci (Lond). 1982 Mar;62(3):295–7.

40. Yamaoka Y, Ohsawa T, Takasan H, Ozawa K. Energy requirement in regenerative and atrophic processes of the liver in man and other mammals. Surg Gynecol Obstet. 1974 Aug;139(2):234–40.

41. Ozawa K, Kamiyama Y, Kimura K, Ukikusa M, Kono Y, Yamato T, et al. The effects of heterologous liver cross-hemodialysis on adenylate energy charge of the remnant liver after major hepatic resection. Artif Organs. 1982 Nov;6(4):447–52.

42. Corbin IR, Buist R, Volotovskyy V, Peeling J, Zhang M, Minuk GY. Regenerative activity and liver function following partial hepatectomy in the rat using (31)P-MR spectroscopy. Hepatology. 2002 Aug;36(2):345–53.

43. Mann D V, Lam WWM, Hjelm NM, So NMC, Yeung DKW, Metreweli C, et al. Metabolic control patterns in acute phase and regenerating human liver determined in vivo by 31-phosphorus magnetic resonance spectroscopy. Ann Surg. 2002 Mar;235(3):408–16.

44. Koyama H, Kurokawa T, Nonami T, Nakao A, Sugiyama S. Increases in the Mitochondrial DNA Replication and Transcription in the Remnant Liver of Rats. 1998;861(243):858–61.

45. Sun Y, Deng X, Li W, Yan Y, Wei H, Jiang Y, et al. Liver proteome analysis of adaptive response in rat immediately after partial hepatectomy. Proteomics. 2007 Dec;7(23):4398–407.

46. Nagino M, Tanaka M, Nishikimi M, Nimura Y, Kubota H, Kanai M. Stimulated Rat Liver Mitochondrial Biogenesis after Partial Hepatectomy. Cancer Res. 1989;49(17):4913–8.

47. Cao H, Yu J, Xu W, Jia X, Yang J, Pan Q, et al. Proteomic analysis of regenerating mouse liver following 50% partial hepatectomy. Proteome Sci. 2009 Jan;7(48):doi: 10.1186/1477-5956-7-48.

48. Guerrieri F, Nicoletti C, Adorisio E, Caraccio G, Leonetti P, Zanotti F, et al. Correlation between decreased expression of mitochondrial F0F1-ATP synthase and low regenerating capability of the liver after partial hepatectomy in hypothyroid rats. J Bioenerg Biomembr. 2000 May;32(2):183–91.

49. Moro L, Marra E, Capuano F, Greco M. Thyroid hormone treatment of hypothyroid rats restores the regenerative capacity and the mitochondrial membrane permeability properties of the liver after partial hepatectomy. Endocrinology. 2004 Nov;145(11):5121–8.

50. Khiati S, Baechler SA, Factor VM, Zhang H, Huang S-YN, Dalla Rosa I, et al. Lack of mitochondrial topoisomerase I (TOP1mt) impairs liver regeneration. Proc Natl Acad Sci U S A. 2015 Sep 8;112(36):11282–7.

51. Yoshioka S, Miyazaki M, Shimizu H, Ito H, Nakagawa K, Ambiru S, et al. Hepatic venous hemoglobin oxygen saturation predicts regenerative status of remnant liver after partial hepatectomy in rats. Hepatology. 1998 May;27(5):1349–53.

52. Satoh S, Tanaka A, Hatano E, Inomoto T, Iwata S, Kitai T, et al. Energy Metabolism and Regeneration in Transgenic Mouse Liver Expressing Creatine Kinase After Major Hepatectomy. Gastroenterology. 1996;110(4):1166–74.

53. Zhong Z, Theruvath TP, Currin RT, Waldmeier PC, Lemasters JJ. NIM811, a mitochondrial permeability transition inhibitor, prevents mitochondrial depolarization in small-for-size rat liver grafts. Am J Transplant. 2007 May;7(5):1103–11.

54. Auger C, Alhasawi A, Contavadoo M, Appanna VD. Dysfunctional mitochondrial bioenergetics and the pathogenesis of hepatic disorders. Front cell Dev Biol. 2015 Jan;3:40.

55. Nishikawa T, Bellance N, Damm A, Bing H, Zhu Z, Handa K, et al. A switch in the source of ATP production and a loss in capacity to perform glycolysis are hallmarks of hepatocyte failure in advance liver disease. J Hepatol. 2014 Jun;60(6):1203–11.

56. Cortez-Pinto H, Chatham J, Chacko VP, Arnold C, Rashid A, Diehl AM. Alterations in liver ATP homeostasis in human nonalcoholic steatohepatitis: a pilot study. JAMA. 1999 Nov 3;282(17):1659–64.

57. McGill MR, Sharpe MR, Williams CD, Taha M, Curry SC, Jaeschke H. The mechanism underlying acetaminophen-induced hepatotoxicity in humans and mice involves mitochondrial damage and nuclear DNA fragmentation. J Clin Invest. 2012 Apr;122(4):1574–83.

58. Lane M, Boczonadi V, Bachtari S, Gomez-Duran A, Langer T, Griffiths A, et al. Mitochondrial dysfunction in liver failure requiring transplantation. J Inherit Metab Dis. 2016;39(3):427–36.

59. Ukikusa M, Ozawa K, Shimahara Y, Asano M, Nakatani T, Tobe T. Changes in blood ketone body ratio: their significance after major hepatic resection. Arch Surg. 1981 Jun;116(6):781–5.

60. Yamaguchi T, Shimahara Y, Takada Y, Ino K, Mori K, Kobayashi N, et al. Evaluation of ketogenesis in seriously reduced hepatic mitochondrial redox state. An analysis of survivors and non-survivors in critically ill hepatectomized patients. Scand J Gastroenterol. 1992 Jun;27(6):472–8.

61. Yamamoto Y, Ozawa K, Okamoto R, Kiuchi T, Maki A, Lin H, et al. Prognostic implications of postoperative suppression of arterial ketone body ratio: time factor involved in the suppression of hepatic mitochondrial oxidation-reduction state. Surgery. 1990 Mar;107(3):289–94.

62. Kainuma M, Nakashima K, Sakuma I, Kawase M, Komatsu T, Shimada Y, et al. Hepatic venous hemoglobin oxygen saturation predicts liver dysfunction after hepatectomy. Anesthesiology. 1992;76(3):379–86.

63. Ozawa K, Kitamura O, Yamaoka Y, Mizukami T, Kamano T. Quantitative analysis of respiratory enzymes of mitochondria isolated from liver tissue of patients. J Lab Clin Med. 1973 Mar;81(3):379–92.

64. Ueda J, Mori K, Sakai Y, Tanaka A, Katayama T, Maki A, et al. Noninvasive evaluation of cytochrome c oxidase activity of the liver. Its prognostic value for hepatic resection. Arch Surg. 1994;129(3):303–8.

65. Castro O, David E, Ii M, Kumar A, Iii S, Eliza M, et al. Biochemical liver function after partial hepatic resection with or without partial hepatic vascular exclusion. Acta Cir Bras. 2011;26(Suppl 2):120–4.

66. Guerrieri F, Vendemiale G, Grattagliano I, Cocco T, Pellecchia G, Altomare E. Mitochondrial oxidative alterations following partial hepatectomy. Free Radic Biol Med. 1999 Jan;26(1–2):34–41.

67. Pringle JH. Notes on the Arrest of Hepatic Hemorrhage due to Trauma. Ann Surg. 1908;4(4):541–9.

68. Chouillard EK, Gumbs A a, Cherqui D. Vascular clamping in liver surgery: physiology, indications and techniques. Ann Surg Innov Res. 2010 Jan;4:2.

69. Varela AT, Simões AM, Teodoro JS, Duarte F V, Gomes AP, Palmeira CM, et al. Indirubin-3'-oxime prevents hepatic I/R damage by inhibiting GSK-3beta and mitochondrial permeability transition. Mitochondrion. Mitochondria Research Society; 2010 Aug;10(5):456–63.

70. Bahde R, Spiegel HU. Hepatic ischaemia-reperfusion injury from bench to bedside. Br J Surg. 2010;97(10):1461–75.

71. Wilasrusmee C, Siritheptawee S, Kanchanapanjapon S, Sopon P, Vanichanon C, Limpthong W, et al. Ultrastructural changes in cirrhotic and noncirrhotic patients due to hepatectomy. J Hepatobiliary Pancreat Surg. 2004 Jan;11(4):266–71.

72. Schoen JM, Wang HH, Minuk GY, Lautt WW. Shear stress-induced nitric oxide release triggers the liver regeneration cascade. Nitric Oxide. 2001 Jan;5(5):453–64.

73. Allard M-A, Adam R, Bucur P-O, Termos S, Cunha AS, Bismuth H, et al. Posthepatectomy portal vein pressure predicts liver failure and mortality after major liver resection on noncirrhotic liver. Ann Surg. 2013 Nov;258(5):822-9-30.

74. Chen X, Zhai J, Cai X, Zhang Y, Wei L, Shi L, et al. Severity of portal hypertension and prediction of postoperative liver failure after liver resection in patients with Child-Pugh grade A cirrhosis. Br J Surg. 2012 Dec;99(12):1701–10.

75. Demetris AJ, Kelly DM, Eghtesad B, Fontes P, Wallis Marsh J, Tom K, et al. Pathophysiologic observations and histopathologic recognition of the portal hyperperfusion or small-for-size syndrome. Am J Surg Pathol. 2006 Aug;30(8):986–93.

76. Golriz M, Majlesara A, El Sakka S, Ashrafi M, Arwin J, Fard N, et al. Small for Size and Flow (SFSF) syndrome: An alternative description for posthepatectomy liver failure. Clin Res Hepatol Gastroenterol. 2015;40(3):267–75.

77. Lautt WW, Legare DJ, Ezzat WR. Quantitation of the hepatic arterial buffer response to graded changes in portal blood flow. Gastroenterology. 1990 Apr;98(4):1024–8.

78. Akamatsu N, Sugawara Y, Satou S, Mitsui T, Ninomiya R, Komagome M, et al. Hemodynamic changes in the hepatic circulation after the modulation of the splenic circulation in an in vivo human experimental model. Liver Transpl. 2014 Jan;20(1):116–21.

79. Lautt WW. Regulatory processes interacting to maintain hepatic blood flow constancy: Vascular compliance, hepatic arterial buffer response, hepatorenal reflex, liver regeneration, escape from vasoconstriction. Hepatol Res. 2007 Nov;37(11):891–903.

80. Eipel C, Abshagen K, Vollmar B. Regulation of hepatic blood flow: the hepatic arterial buffer response revisited. World J Gastroenterol. 2010 Dec 28;16(48):6046–57.

81. Kostopanagiotou G, Pandazi A, Arkadopoulos N, Theodoraki K, Mystakidou K, Costopanagiotou C, et al. Norepinephrine in Small-For-Size Liver Grafts: An Experimental Study in Pigs. J Surg Res. 2007;141(2):257–61.

82. Zhu X, Fung JJ, Nakagawa S, Wang LF, Irefin S, Cocieru A, et al. Elevated catecholamines and hepatic artery vasospasm in porcine small-for-size liver graft. J Surg Res. 2012;174(1):157–65.

83. Dold S, Richter S, Kollmar O, Von Heesen M, Scheuer C, Laschke MW, et al. Portal hyperperfusion after extended hepatectomy does not induce a hepatic arterial buffer response (HABR) but impairs mitochondrial redox state and hepatocellular oxygenation. PLoS One. 2015;10(11):e0141877.

84. Xiang L, Huang L, Wang X, Zhao Y, Liu Y, Tan J. How much portal vein flow is too much for liver remnant in a stable porcine model? Transplant Proc. 2016;48(1):234–41.

85. Ninomiya M, Shirabe K, Terashi T, Ijichi H, Yonemura Y, Harada N, et al. Deceleration of regenerative response improves the outcome of rat with massive hepatectomy. Am J Transplant. 2010;10(7):1580–7.

86. McGill MR, Staggs VS, Sharpe MR, Lee WM, Jaeschke H. Serum mitochondrial biomarkers and damage-associated molecular patterns are higher in acetaminophen overdose patients with poor outcome. Hepatology. 2014 Oct;60(4):1336–45.

87. Helbling D, Buchaklian A, Wang J, Wong L-J, Dimmock D. Reduced mitochondrial DNA content and heterozygous nuclear gene mutations in patients with acute liver failure. J Pediatr Gastroenterol Nutr. 2013 Oct;57(4):438–43.

88. Ratziu V, Bellentani S, Cortez-Pinto H, Day C, Marchesini G. A position statement on NAFLD/NASH based on the EASL 2009 special conference. J Hepatol. 2010 Aug;53(2):372–84.

89. Mittal S, El-Serag HB, Sada YH, Kanwal F, Duan Z, Temple S, et al. Hepatocellular Carcinoma in the Absence of Cirrhosis in US Veterans is Associated with Non-Alcoholic Fatty Liver Disease. Clin Gastroenterol Hepatol. 2015 Jul 18;14(124–31).

90. Adam R, De Gramont A, Figueras J, Guthrie A, Kokudo N, Kunstlinger F, et al. The oncosurgery approach to managing liver metastases from colorectal cancer: a multidisciplinary international consensus. Oncologist. 2012 Jan;17(10):1225–39.

91. Gusdon AM, Song K-X, Qu S. Nonalcoholic Fatty liver disease: pathogenesis and therapeutics from a mitochondria-centric perspective. Oxid Med Cell Longev. 2014 Jan;2014:637027.

92. Pessayre D, Fromenty B. NASH: a mitochondrial disease. J Hepatol. 2005

Jun;42(6):928–40.

93. Grattagliano I, de Bari O, Bernardo TC, Oliveira PJ, Wang DQ-H, Portincasa P. Role of mitochondria in nonalcoholic fatty liver disease--from origin to propagation. Clin Biochem. The Canadian Society of Clinical Chemists; 2012 Jun;45(9):610–8.

94. Pirola CJ, Gianotti TF, Burgueño AL, Rey-Funes M, Loidl CF, Mallardi P, et al. Epigenetic modification of liver mitochondrial DNA is associated with histological severity of nonalcoholic fatty liver disease. Gut. 2013 Sep;62(9):1356–63.

95. Reiniers MJ, van Golen RF, van Gulik TM, Heger M. Reactive oxygen and nitrogen species in steatotic hepatocytes: a molecular perspective on the pathophysiology of ischemia-reperfusion injury in the fatty liver. Antioxid Redox Signal. 2014 Sep 1;21(7):1119–42.

96. Kaur J. A Comprehensive Review on Metabolic Syndrome. Cardiol Res Pract. 2014 Jan;2014:1–21.

97. Guicciardi ME, Malhi H, Mott JL, Gores GJ. Apoptosis and necrosis in the liver. Compr Physiol. 2013 Apr;3(2):977–1010.

98. Koliaki C, Szendroedi J, Kaul K, Jelenik T, Nowotny P, Jankowiak F, et al. Adaptation of hepatic mitochondrial function in humans with non-alcoholic fatty liver is lost in steatohepatitis. Cell Metab. 2015 May 5;21(5):739–46.

99. Verbeek J, Lannoo M, Pirinen E, Ryu D, Spincemaille P, Vander Elst I, et al. Roux-en-y gastric bypass attenuates hepatic mitochondrial dysfunction in mice with non-alcoholic steatohepatitis. Gut. 2015 Apr;64(4):673–83.

100. Teodoro JS, Duarte FV, Gomes AP, Varela AT, Peixoto FM, Rolo AP, et al. Berberine reverts hepatic mitochondrial dysfunction in high-fat fed rats: a possible role for SirT3 activation. Mitochondrion. Elsevier B.V.; 2013 Nov;13(6):637–46.

101. Perry RJ, Zhang D, Zhang X-M, Boyer JL, Shulman GI. Controlled-release mitochondrial protonophore reverses diabetes and steatohepatitis in rats. Science. 2015 Mar 13;347(6227):1253–6.

102. Veteläinen R, Bennink RJ, van Vliet AK, van Gulik TM. Mild steatosis impairs functional recovery after liver resection in an experimental model. Br J Surg. 2007 Aug;94(8):1002–8.

103. Veteläinen R, van Vliet AK, van Gulik TM. Severe steatosis increases hepatocellular injury and impairs liver regeneration in a rat model of partial hepatectomy. Ann Surg. 2007 Jan;245(1):44–50.

104. Sydor S, Gu Y, Schlattjan M, Bechmann LP, Rauen U, Best J, et al. Steatosis does not impair liver regeneration after partial hepatectomy. Lab Investig. Nature Publishing Group; 2012;93(1):20–30.

105. Garnol T, Kučera O, Staňková P, Lotková H, Červinková Z. Does Simple

Steatosis Affect Liver Regeneration after Partial Hepatectomy in Rats? Acta medica (Hradec Králové) / Univ Carolina, Fac Medica Hradec Králové. 2016;59(2):35–42.

106. Veteläinen R, van Vliet A, Gouma DJ, van Gulik TM. Steatosis as a risk factor in liver surgery. Ann Surg. 2007 Jan;245(1):20–30.

107. Varela AT, Rolo AP, Palmeira CM. Fatty liver and ischemia/reperfusion: are there drugs able to mitigate injury? Curr Med Chem. 2011 Jan;18(32):4987–5002.

108. Rolo AP, Teodoro JS, Peralta C, Rosello-Catafau J, Palmeira CM. Prevention of I/R injury in fatty livers by ischemic preconditioning is associated with increased mitochondrial tolerance: the key role of ATPsynthase and mitochondrial permeability transition. Transpl Int. 2009 Nov;22(11):1081–90.

109. Cauchy F, Zalinski S, Dokmak S, Fuks D, Farges O, Castera L, et al. Surgical treatment of hepatocellular carcinoma associated with the metabolic syndrome. Br J Surg. 2013 Jan;100(1):113–21.

110. Parkin E, O'Reilly DA, Adam R, Kaiser GM, Laurent C, Elias D, et al. The effect of hepatic steatosis on survival following resection of colorectal liver metastases in patients without preoperative chemotherapy. HPB (Oxford). 2013 Jun;15(6):463–72.

111. de Meijer VE, Kalish BT, Puder M, Ijzermans JNM. Systematic review and meta-analysis of steatosis as a risk factor in major hepatic resection. Br J Surg. 2010 Sep;97(9):1331–9.

112. Kooby DA, Fong Y, Suriawinata A, Gonen M, Allen PJ, Klimstra DS, et al. Impact of steatosis on perioperative outcome following hepatic resection. J Gastrointest Surg. 2003 Dec;7(8):1034–44.

113. McCormack L, Petrowsky H, Jochum W, Furrer K, Clavien P-A. Hepatic steatosis is a risk factor for postoperative complications after major hepatectomy: a matched case-control study. Ann Surg. 2007 Jun;245(6):923–30.

114. Reddy SK, Marsh JW, Varley PR, Mock BK, Chopra KB, Geller DA, et al. Underlying steatohepatitis, but not simple hepatic steatosis, increases morbidity after liver resection: A case-control study. Hepatology. 2012 Dec 14;56(6):2221–30.

115. Martins J, Alexandrino H, Oliveira R, Cipriano MA, Falcão D, Ferreira L, et al. Sinusoidal dilation increases the risk of complications in hepatectomy for CRCLM – Protective effect of bevacizumab and diabetes mellitus, serum gamma-glutamyltranspeptidase as predictive factor. Eur J Surg Oncol. 2016 Feb 24;42(5):713–21.

116. Vauthey J-N, Pawlik TM, Ribero D, Wu T-T, Zorzi D, Hoff PM, et al. Chemotherapy regimen predicts steatohepatitis and an increase in 90-day mortality after surgery for hepatic colorectal metastases. J Clin Oncol. 2006 May 1;24(13):2065–72.

117. Neal CP, Mann CD, Pointen E, McGregor A, Garcea G, Metcalfe MS, et al. Influence of hepatic parenchymal histology on outcome following right hepatic trisectionectomy. J Gastrointest Surg. 2012 Nov;16(11):2064–73.

118. Wiggans MG, Lordan JT, Shahtahmassebi G, Aroori S, Bowles MJ, Stell DA. The Interaction between Diabetes, Body Mass Index, Hepatic Steatosis, and Risk of Liver Resection: Insulin Dependent Diabetes Is the Greatest Risk for Major Complications. HPB Surg. 2014 Jan;2014:1–10.

119. Behrns KE, Tsiotos GG, DeSouza NF, Krishna MK, Ludwig J, Nagorney DM. Hepatic steatosis as a potential risk factor for major hepatic resection. J Gastrointest Surg. 1998;2(3):292–8.

120. Gomez D, Malik HZ, Bonney GK, Wong V, Toogood GJ, Lodge JP a, et al. Steatosis predicts postoperative morbidity following hepatic resection for colorectal metastasis. Br J Surg. 2007 Nov;94(11):1395–402.

121. Schuppan D, Afdhal NH. Liver cirrhosis. Lancet. 2008 Mar 8;371(9615):838–51.

122. Cescon M, Cucchetti A, Grazi GL, Ferrero A, Viganò L, Ercolani G, et al. Indication of the extent of hepatectomy for hepatocellular carcinoma on cirrhosis by a simple algorithm based on preoperative variables. Arch Surg. 2009 Jan;144(1):57–63; discussion 63.

123. Sugiyama Y, Ishizaki Y, Imamura H, Sugo H, Yoshimoto J, Kawasaki S. Effects of intermittent Pringle ' s manoeuvre on cirrhotic compared with normal liver. Br J Surg. 2010;97(7):1062–9.

124. Hashimoto M, Watanabe G. Functional restoration of cirrhotic liver after partial hepatectomy in the rat. Hepatogastroenterology. 2005 Jan;52(63):897–902.

125. Froomes PR, Morgan DJ, Smallwood RA, Angus PW. Comparative effects of oxygen supplementation on theophylline and acetaminophen clearance in human cirrhosis. Gastroenterology. 1999 Apr;116(4):915–20.

126. Yang S, Tan TMC, Wee A, Leow CK. Mitochondrial respiratory function and antioxidant capacity in normal and cirrhotic livers following partial hepatectomy. Cell Mol Life Sci. 2004;61(2):220–9.

127. Arvaniti V, D'Amico G, Fede G, Manousou P, Tsochatzis E, Pleguezuelo M, et al. Infections in patients with cirrhosis increase mortality four-fold and should be used in determining prognosis. Gastroenterology. 2010 Oct;139(4):1246–56, 1256-5.

128. Mann D V, Lam WW, Hjelm NM, So NM, Yeung DK, Metreweli C, et al. Human liver regeneration: hepatic energy economy is less efficient when the organ is diseased. Hepatology. 2001 Sep;34(3):557–65.

129. Folprecht G, Grothey a, Alberts S, Raab H-R, Köhne C-H. Neoadjuvant treatment of unresectable colorectal liver metastases: correlation between tumour response and resection rates. Ann Oncol. 2005 Aug;16(8):1311–9.

130. Nordlinger B, Sorbye H, Glimelius B, Poston GJ, Schlag PM, Rougier P, et al.

Perioperative chemotherapy with FOLFOX4 and surgery versus surgery alone for resectable liver metastases from colorectal cancer (EORTC Intergroup trial 40983): a randomised controlled trial. Lancet. 2008 Mar;371(9617):1007–16.

131. Lentz F, Tran A, Rey E, Pons G, Tréluyer J-M. Pharmacogenomics of fluorouracil, irinotecan, and oxaliplatin in hepatic metastases of colorectal cancer: clinical implications. Am J Pharmacogenomics. 2005 Jan;5(1):21–33.

132. Allard MA, Sebagh M, Baillie G, Lemoine A, Dartigues P, Faitot F, et al. Comparison of complete pathologic response and hepatic injuries between hepatic arterial infusion and systemic administration of oxaliplatin in patients with colorectal liver metastases. Ann Surg Oncol. 2015 Jun;22(6):1925–32.

133. Karoui M, Penna C, Amin-Hashem M, Mitry E, Benoist S, Franc B, et al. Influence of preoperative chemotherapy on the risk of major hepatectomy for colorectal liver metastases. Ann Surg. 2006 Jan;243(1):1–7.

134. Takamoto T, Hashimoto T, Sano K, Maruyama Y, Inoue K, Ogata S, et al. Recovery of liver function after the cessation of preoperative chemotherapy for colorectal liver metastasis. Ann Surg Oncol. 2010 Oct;17(10):2747–55.

135. Labbe G, Pessayre D, Fromenty B. Drug-induced liver injury through mitochondrial dysfunction: mechanisms and detection during preclinical safety studies. Fundam Clin Pharmacol. 2008 Aug;22(4):335–53.

136. Rubbia-Brandt L, Lauwers GY, Wang H, Majno PE, Tanabe K, Zhu AX, et al. Sinusoidal obstruction syndrome and nodular regenerative hyperplasia are frequent oxaliplatin-associated liver lesions and partially prevented by bevacizumab in patients with hepatic colorectal metastasis. Histopathology. 2010 Mar;56(4):430–9.

137. Fan CQ, Crawford JM. Sinusoidal Obstruction Syndrome (Hepatic Veno-Occlusive Disease). J Clin Exp Hepatol. Elsevier Ltd; 2014;4(4):332–46.

138. Aloia T, Sebagh M, Plasse M, Karam V, Lévi F, Giacchetti S, et al. Liver histology and surgical outcomes after preoperative chemotherapy with fluorouracil plus oxaliplatin in colorectal cancer liver metastases. J Clin Oncol. 2006 Nov 1;24(31):4983–90.

139. Narita M, Oussoultzoglou E, Fuchshuber P, Chenard M-P, Rosso E, Yamamoto K, et al. Prolonged portal triad clamping increases postoperative sepsis after major hepatectomy in patients with sinusoidal obstruction syndrome and/or steatohepatitis. World J Surg. 2012 Aug;36(8):1848–57.

140. Narita M, Oussoultzoglou E, Fuchshuber P, Pessaux P, Chenard M-P, Rosso E, et al. What is a safe future liver remnant size in patients undergoing major hepatectomy for colorectal liver metastases and treated by intensive preoperative chemotherapy? Ann Surg Oncol. 2012 Aug;19(8):2526–38.

141. Nakano H, Oussoultzoglou E, Rosso E, Casnedi S, Chenard-Neu M-P, Dufour P, et al. Sinusoidal injury increases morbidity after major hepatectomy in patients with colorectal liver metastases receiving preoperative chemotherapy. Ann Surg.

2008 Jan;247(1):118–24.

142. Narita M, Oussoultzoglou E, Chenard MP, Rosso E, Casnedi S, Pessaux P, et al. Sinusoidal obstruction syndrome compromises liver regeneration in patients undergoing two-stage hepatectomy with portal vein embolization. Surg Today. 2011;41(1):7–17.

143. de Baere T, Teriitehau C, Deschamps F, Catherine L, Rao P, Hakime A, et al. Predictive Factors for Hypertrophy of the Future Remnant Liver After Selective Portal Vein Embolization. Ann Surg Oncol. 2010 Mar 17;17(8):2081–9.

144. Tabassum H, Waseem M, Parvez S, Qureshi MI. Oxaliplatin-induced Oxidative Stress Provokes Toxicity in Isolated Rat Liver Mitochondria. Arch Med Res. 2015 Nov 9;6(8):597–603.

145. Rosen M, Figliomeni M, Simpkins H. The interaction of platinum antitumour drugs with mouse liver mitochondria. Int J Exp Pathol. 1992 Feb;73(1):61–74.

146. Gourdier I, Crabbe L, Andreau K, Pau B, Kroemer G. Oxaliplatin-induced mitochondrial apoptotic response of colon carcinoma cells does not require nuclear DNA. Oncogene. 2004;(23):7449–57.

147. Schiffer E, Frossard J-L, Rubbia-Brandt L, Mentha G, Pastor CM. Hepatic regeneration is decreased in a rat model of sinusoidal obstruction syndrome. J Surg Oncol. 2009 Jun 1;99(7):439–46.

148. Robinson SM, Mann J, Vasilaki a, Mathers J, Burt a D, Oakley F, et al. Pathogenesis of FOLFOX induced sinusoidal obstruction syndrome in a murine chemotherapy model. J Hepatol. European Association for the Study of the Liver; 2013 Aug;59(2):318–26.

149. Neuhaus P, Jonas S, Bechstein WO. Extended Resections for Hilar Cholangiocarcinoma. Ann Surg. 1999;230(6):808–19.

150. Yokoyama Y, Ebata T, Igami T, Sugawara G. Predictive power of prothrombin time and serum total bilirubin for postoperative mortality after major hepatectomy with extrahepatic bile duct resection. Surgery. Mosby, Inc.; 2011;155(3):504–11.

151. Iacono C, Ruzzenente A, Campagnaro T, Bortolasi L, Valdegamberi A, Guglielmi A. Role of preoperative biliary drainage in jaundiced patients who are candidates for pancreatoduodenectomy or hepatic resection: highlights and drawbacks. Ann Surg. 2013 Mar;257(2):191–204.

152. Baton O, Azoulay D, Adam DVR, Castaing D. Major hepatectomy for hilar cholangiocarcinoma type 3 and 4: prognostic factors and longterm outcomes. J Am Coll Surg. 2007 Feb;204(2):250–60.

153. Igami T, Nishio H, Ebata T, Yokoyama Y, Sugawara G, Nimura Y, et al. Surgical treatment of hilar cholangiocarcinoma in the “new era”: the Nagoya University experience. J Hepatobiliary Pancreat Sci. 2010 Jul;17(4):449–54.

154. Wiggers JK, Groot Koerkamp B, Cieslak KP, Doussot A, van Klaveren D, Allen

PJ, et al. Postoperative Mortality after Liver Resection for Perihilar Cholangiocarcinoma: Development of a Risk Score and Importance of Biliary Drainage of the Future Liver Remnant. J Am Coll Surg. 2016 Aug;223(2):321–331.e1.

155. Palmeira CM, Rolo AP. Mitochondrially-mediated toxicity of bile acids. Toxicology. 2004;203(1–3):1–15.

156. Rolo AP, Oliveira PJ, Moreno JM, Palmeira CM. Bile Acids Affect Liver Mitochondrial Bioenergetics : Possible Relevance for Cholestasis Therapy. 2000;57(1):177–85.

157. Schulz S, Schmitt S, Wimmer R, Aichler M, Eisenhofer S, Lichtmannegger J, et al. Progressive stages of mitochondrial destruction caused by cell toxic bile salts. BBA - Biomembr. Elsevier B.V.; 2013;81(9):2121–33.

158. Arduini A, Serviddio G, Escobar J, Tormos AM, Bellanti F, Viña J, et al. Mitochondrial biogenesis fails in secondary biliary cirrhosis in rats leading to mitochondrial DNA depletion and deletions. Am J Physiol Gastrointest Liver Physiol. 2011 Jul;301(1):G119-27.

159. Kanai M, Tanaka M, Nimura Y, Nagino M, Katoh T, Ozawa T. Mitochondrial dysfunction in the non-obstructed lobe of rat liver after selective biliary obstruction. Hepatogastroenterology. 1992 Oct;39(5):385–91.

160. Krähenbühl L, Schäfer M, Krähenbühl S. Reversibility of hepatic mitochondrial damage in rats with long-term cholestasis. J Hepatol. 1998 Jun;28(6):1000–7.

161. Mann D V, Lam WWM, Magnus Hjelm N, So NMC, Yeung DKW, Metreweli C, et al. Biliary drainage for obstructive jaundice enhances hepatic energy status in humans: a 31-phosphorus magnetic resonance spectroscopy study. Gut. 2002 Jan;50(1):118–22.

162. Rahbari NN, Garden OJ, Padbury R, Brooke-Smith M, Crawford M, Adam R, et al. Posthepatectomy liver failure: a definition and grading by the International Study Group of Liver Surgery (ISGLS). Surgery. 2011 May;149(5):713–24.

163. Siu J, Mccall J, Connor S. Systematic review of pathophysiological changes following hepatic resection. HPB (Oxford). 2014;16(January 2000):407–21.

164. Hoekstra LT, de Graaf W, Nibourg GAA, Heger M, Bennink RJ, Stieger B, et al. Physiological and Biochemical Basis of Clinical Liver Function Tests. Ann Surg. 2013 Jan;257(1):27–36.

165. Balzan S, Belghiti J, Farges O, Ogata S, Sauvanet A, Delefosse D, et al. The "50-50 criteria" on postoperative day 5: an accurate predictor of liver failure and death after hepatectomy. Ann Surg. 2005 Dec;242(6):824–8, NaN-9.

166. Kim SH, Kang DR, Lee JG, Kim DY, Ahn SH, Han K-H, et al. Early predictor of mortality due to irreversible posthepatectomy liver failure in patients with hepatocellular carcinoma. World J Surg. 2013 May;37(5):1028–33.

167. Bernal W. Lactate is important in determining prognosis in acute liver failure. J

Hepatol. European Association for the Study of the Liver; 2010 Jul;53(1):209–10.

168. Rady MY. Bench-to-bedside review: Resuscitation in the emergency department. Crit Care. 2005 Apr;9(2):170–6.

169. Detry O, Gaspar Y, Cheramy-Bien J-P, Drion P, Meurisse M, Defraigne J-O. A modified surgical model of fulminant hepatic failure in the rat. J Surg Res. Elsevier Ltd; 2013 May 1;181(1):85–90.

170. Watanabe I, Mayumi T, Arishima T, Takahashi H, Takezawa J, Medicine E, et al. Hyperlactemia can predict the Prognosis of Liver Resection. Shock. 2007;28(1):35–8.

171. Vibert E, Boleslawski E, Cosse C, Adam R, Castaing D, Cherqui D, et al. Arterial Lactate Concentration at the End of an Elective Hepatectomy Is an Early Predictor of the Postoperative Course and a Potential Surrogate of Intraoperative Events. Ann Surg. 2015 Nov;262(5):787–93.

172. Theodoraki K, Arkadopoulos N, Fragulidis G, Voros D, Karapanos K, Markatou M, et al. Transhepatic lactate gradient in relation to liver ischemia/reperfusion injury during major hepatectomies. Liver Transpl. 2006 Dec;12(12):1825–31.

173. Hallet J, Karanicolas PJ, Zih FSW, Cheng E, Wong J, Hanna S, et al. Hypophosphatemia and recovery of post-hepatectomy liver insufficiency. Hepatobiliary Surg Nutr. 2016 Jun;5(3):217–24.

174. Squires MH, Dann GC, Lad NL, Fisher SB, Martin BM, Kooby D a, et al. Hypophosphataemia after major hepatectomy and the risk of post-operative hepatic insufficiency and mortality: an analysis of 719 patients. HPB (Oxford). 2014 Oct;16(10):884–91.

175. Salem RR, Tray K. Hepatic resection-related hypophosphatemia is of renal origin as manifested by isolated hyperphosphaturia. Ann Surg. 2005 Feb;241(2):343–8.

176. Nakamura K, Onitsuka T, Yano M, Yano Y. Study in three different types of cardiopulmonary bypass on arterial ketone body ratio: its prognostic implication and participation of body temperature. Interact Cardiovasc Thorac Surg. 2003 Mar;2(1):25–9.

177. Yan L-N, Chen X-L, Li Z-H, Li B, Lu S-C, Wen T-F, et al. Perioperative management of primary liver cancer. World J Gastroenterol. 2007 Apr 7;13(13):1970–4.

178. Higashi Shushi, Tabata Naoto, Kondo Kazu-hiro, Kai Masahiro, Miyamoto Koji, Maeda Yorio ST. A Predominant Increase of Arterial Beta-Hydroxybutyrate Concentration during Partial Hepatectomies in Patients with Impaired Indocyanine Green Clearance Test. J Surg Res. 1999;81(2):243–8.

179. Perera MTPR, Richards DA, Silva MA, Ahmed N, Neil DA, Murphy N, et al. Comparison of energy metabolism in liver grafts from donors after circulatory death and donors after brain death during cold storage and reperfusion. Br J Surg.

2014;101(7):775–83.

180. Haugaa H, Thorgersen EB, Pharo A, Boberg KM, Foss A, Line PD, et al. Early bedside detection of ischemia and rejection in liver transplants by microdialysis. Liver Transplant. 2012;18(7):839–49.

181. Isaksson B, D'souza MA, Jersenius U, Ungerstedt J, Lundell L, Permert J, et al. Continuous assessment of intrahepatic metabolism by microdialysis during and after portal triad clamping. J Surg Res. 2011 Aug;169(2):214–9.

182. Winbladh A, Björnsson B, Trulsson L, Offenbartl K, Gullstrand P, Sandström P. Ischemic preconditioning prior to intermittent Pringle maneuver in liver resections. J Hepatobiliary Pancreat Sci. 2012 Mar;19(2):159–70.

183. Chu MJJ, Phillips ARJ, Hosking AWG, Macdonald JR, Bartlett ASJR, Hickey AJR. Hepatic Mitochondrial Function Analysis Using Needle Liver Biopsy Samples. PLoS One. 2013;8(10):243–8.

184. Shi Q, Yang X, Mattes WB, Mendrick DL, Harrill AH, Beger RD. Circulating mitochondrial biomarkers for drug-induced liver injury. Biomark Med. 2015 Jan;9(11):1215–23.

185. McGill MR, Li F, Sharpe MR, Williams CD, Curry SC, Ma X, et al. Circulating acylcarnitines as biomarkers of mitochondrial dysfunction after acetaminophen overdose in mice and humans. Arch Toxicol. 2014 Mar;88(2):391–401.

186. Bernal W, Auzinger G, Dhawan A, Wendon J. Acute liver failure. Lancet. Elsevier Ltd; 2010 Jul 17;376(9736):190–201.

187. Schomaker S, Warner R, Bock J, Johnson K, Potter D, Winkle J Van, et al. Assessment of Emerging Biomarkers of Liver Injury in Human Subjects. Toxicol Sci. 2013;132(2):276–83.

188. Kellersmann R, Gassel H-J, Bühler C, Thiede A, Timmermann W. Application of Molecular Adsorbent Recirculating System in patients with severe liver failure after hepatic resection or transplantation: initial single-centre experiences. Liver. 2002;22(Suppl 2):56–8.

189. Barshes NR, Gay a N, Williams B, Patel AJ, Awad SS. Support for the acutely failing liver: a comprehensive review of historic and contemporary strategies. J Am Coll Surg. 2005 Sep;201(3):458–76.

190. Narita M, Oussoultzoglou E, Bachellier P, Jaeck D, Uemoto S. Post-hepatectomy liver failure in patients with colorectal liver metastases. Surg Today. 2015 Oct 29;45(10):1218–26.

191. Bailey SM, Robinson G, Pinner A, Chamlee L, Ulasova E, Pompilius M, et al. S-adenosylmethionine prevents chronic alcohol-induced mitochondrial dysfunction in the rat liver. Am J Physiol Gastrointest Liver Physiol. 2006 Nov;291(5):G857-67.

192. Brown JM, Kuhlman C, Terneus M V, Labenski MT, Lamyaithong AB, Ball JG, et al. S-adenosyl-l-methionine protection of acetaminophen mediated oxidative

stress and identification of hepatic 4-hydroxynonenal protein adducts by mass spectrometry. Toxicol Appl Pharmacol. 2014 Dec 1;281(2):174–84.

193. Jeon BR, Lee SM. S-adenosylmethionine protects post-ischemic mitochondrial injury in rat liver. J Hepatol. 2001 Mar;34(3):395–401.

194. Su Z-R, Cui Z-L, Ma J-L, Li J-S, Ge Y, Yu J-H, et al. Beneficial effects of S-adenosyl-L-methionine on post-hepatectomy residual liver function: a prospective, randomized, controlled clinical trial. Hepatogastroenterology. Jan;60(125):1136–41.

195. Liu G, Wang W, Jia W, Xu G, Ma J, Ge Y, et al. Protective effect of S-adenosylmethionine on hepatic ischemia-reperfusion injury during hepatectomy in HCC patients with chronic HBV infection. World J Surg Oncol. 2014 Jan;12:27.

196. Zhang P, Ma D, Wang Y, Zhang M, Qiang X, Liao M, et al. Berberine protects liver from ethanol-induced oxidative stress and steatosis in mice. Food Chem Toxicol. Elsevier Ltd; 2014;74:225–32.

197. Yu W, Sheng M, Xu R, Yu J, Cui K, Tong J, et al. Berberine protects human renal proximal tubular cells from hypoxia/reoxygenation injury via inhibiting endoplasmic reticulum and mitochondrial stress pathways. J Transl Med. Journal of Translational Medicine; 2013 Jan;11(1):24.

198. Yan H-M, Xia M-F, Wang Y, Chang X-X, Yao X-Z, Rao S-X, et al. Efficacy of Berberine in Patients with Non-Alcoholic Fatty Liver Disease. PLoS One. 2015;10(8):e0134172.

199. LaBrecque DR, Steele G, Fogerty S, Wilson M, Barton J. Purification and physical-chemical characterization of hepatic stimulator substance. Hepatology. 7(1):100–6.

200. Gandhi CR. Augmenter of liver regeneration. Fibrogenesis Tissue Repair. 2012;5(1):10.

201. Thirunavukkarasu C, Wang LF, Harvey SAK, Watkins SC, Chaillet JR, Prelich J, et al. Augmenter of liver regeneration: an important intracellular survival factor for hepatocytes. J Hepatol. 2008 Apr;48(4):578–88.

202. Jiang S, Li W, An W. Adenoviral Gene Transfer of Hepatic Stimulator Substance Confers Resistance Against Hepatic Ischemia – Reperfusion Injury by Improving Mitochondrial Function. 2013;456(April):443–56.

203. Li S, Tang Z, Yu H, Li W, Jiang Y, Wang Y, et al. Administration of Naked Plasmid Encoding Hepatic Stimulator Substance by Hydrodynamic Tail Vein Injection Protects Mice from Hepatic Failure by Suppressing the Mitochondrial Permeability Transition. J Pharmacol Exp Ther. 2011;338(3):750–7.

204. Polimeno L, Capuano F, Marangi LC, Margiotta M, Lisowsky T, Ierardi E, et al. The augmenter of liver regeneration induces mitochondrial gene expression in rat liver and enhances oxidative phosphorylation capacity of liver mitochondria. Dig

Liver Dis. 32(6):510–7.

205. Tang C, Lin H, Wu Q, Zhang Y, Bie P, Yang J. Recombinant human augmenter of liver regeneration protects hepatocyte mitochondrial DNA in rats with obstructive jaundice. J Surg Res. Elsevier Inc; 2015 Jun 1;196(1):90–101.

206. Naoumov N V. Cyclophilin inhibition as potential therapy for liver diseases. J Hepatol. 2014 Nov;61(5):1166–74.

207. Šileikytė J, Forte M. Shutting down the pore: The search for small molecule inhibitors of the mitochondrial permeability transition. Biochim Biophys Acta. 2016 Aug;1857(8):1197–202.

208. Lin C-W, Chen Y-S, Lin C-C, Chen Y-J, Lo G-H, Lee P-H, et al. Amiodarone as an autophagy promoter reduces liver injury and enhances liver regeneration and survival in mice after partial hepatectomy. Sci Rep. 2015;5:15807.

209. Meguro M, Mizuguchi T, Kawamoto M, Nakamura Y, Ota S, Kukita K, et al. Continuous monitoring of central venous oxygen saturation predicts postoperative liver dysfunction after liver resection. Surgery. 2013 Aug;154(2):351–62.

210. Richardson AJ, Laurence JM, Lam VWT. Portal triad clamping versus other methods of vascular control in liver resection: a systematic review and meta-analysis. 2012;14(6):355–64.

211. Hoekstra LT, van Trigt JD, Reiniers MJ, Busch OR, Gouma DJ, van Gulik TM. Vascular occlusion or not during liver resection: the continuing story. Dig Surg. 2012 Jan;29(1):35–42.

212. Lesurtel M, Lehmann K, Rougemont O De, Clavien P. Clamping techniques and protecting strategies in liver surgery. HPB (Oxford). 2009;11(4):290–5.

213. Nonami T, Asahi K, Harada A, Nakao A, Takagi H. Effect of hyperdynamic circulatory support on hepatic hemodynamics, oxygen supply and demand after massive hepatectomy. Surgery. 1991 Mar;109(3 Pt 1):277–83.

214. Taurà P, Fuster J, Mercadal J, Martinez-Palli G, Fondevila C, Blasi A, et al. The use of beta-adrenergic drugs improves hepatic oxygen metabolism in cirrhotic patients undergoing liver resection. J Hepatol. European Association for the Study of the Liver; 2010 Mar;52(3):340–7.

215. Kawano Y, Akimaru K, Takubo K, Matsumoto K, Yoshida H, Mamada Y, et al. Jejunectomy can reduce excessively elevated portal pressure after major hepatectomy in beagle dogs. J Surg Res. 2006 Jan;130(1):24–33.

216. Glanemann M, Eipel C, Nussler AK, Vollmar B, Neuhaus P. Hyperperfusion syndrome in small-for-size livers. Eur Surg Res. Jan;37(6):335–41.

217. Eipel C, Abshagen K, Ritter J, Cantré D, Menger MD, Vollmar B. Splenectomy improves survival by increasing arterial blood supply in a rat model of reduced-size liver. Transpl Int. 2010 Oct;23(10):998–1007.

218. Zhuang ZG, Xu JR, Qian LJ, Xia Q, Chi JC. Computed tomography perfusion study of Hemodynamic Changes and portal hyperperfusion in a rabbit model of small-for-size liver. Hepatobiliary Pancreat Dis Int. 2012;11(1):74–80.

219. Di Domenico S, Santori G, Traverso N, Balbis E, Furfaro A, Grillo F, et al. Early effects of portal flow modulation after extended liver resection in rat. Dig Liver Dis. 2011 Oct;43(10):814–22.

220. Ito K, Ozasa H, Noda Y, Koike Y, Arii S, Horikawa S. Splenic artery ligation improves remnant liver function in partially hepatectomized rats with ischemia/reperfusion injury. Liver Int. 2007 Apr;27(3):400–7.

221. Bucur PO, Bekheit M, Audebert C, Othman A, Hammad S, Sebagh M, et al. Modulating Portal Hemodynamics With Vascular Ring Allows Efficient Regeneration After Partial Hepatectomy in a Porcine Model. Ann Surg. 2017 Feb 1;(doi: 10.1097/SLA.0000000000002146. [Epub ahead of print]).

222. Taniguchi M, Shimamura T, Suzuki T, Yamashita K, Oura T, Watanabe M, et al. Transient portacaval shunt for a small-for-size graft in living donor liver transplantation. Liver Transpl. 2007 Jun;13(6):932–4.

223. Troisi R, de Hemptinne B. Clinical relevance of adapting portal vein flow in living donor liver transplantation in adult patients. Liver Transpl. 2003 Sep;9(9):S36-41.

224. Famularo S, Nefotyou K, Fotiadis N, Khan N, Foxton M, Khan AZ. Small-for-Size Liver Syndrome: a Case Series with a Proposal for Management Based on Portal Flow Modulation. J Gastrointest Cancer. 2015 Jun;46(2):185–9.

225. Sato Y, Kobayashi T, Nakatsuka H, Yamamoto S, Oya H, Watanabe T, et al. Splenic arterial ligation prevents liver injury after a major hepatectomy by a reduction of surplus portal hypertension in hepatocellular carcinoma patients with cirrhosis. Hepatogastroenterology. 2001;48(39):831–5.

226. Kelly DM, Zhu X, Shiba H, Irefin S, Trenti L, Cocieru A, et al. Adenosine restores the hepatic artery buffer response and improves survival in a porcine model of small-for-size syndrome. Liver Transpl. 2009 Nov;15(11):1448–57.

227. Nagamine K, Kubota T, Togo S, Nagashima Y, Mori M, Shimada H. Beneficial effect of hyperbaric oxygen therapy on liver regeneration after 90% hepatectomy in rats. Eur Surg Res Eur Chir Forschung Rech Chir Eur. Jan;36(6):350–6.

228. Tolentino EC, Castro e Silva O, Zucoloto S, Souza MEJ, Gomes MCJ, Sankarankutty AK, et al. Effect of hyperbaric oxygen on liver regeneration in a rat model. Transplant Proc. Jan;38(6):1947–52.

229. Sgarbi G, Giannone F, Casalena GA, Baracca A, Baldassare M, Longobardi P, et al. Hyperoxia fully protects mitochondria of explanted livers. J Bioenerg Biomembr. 2011 Dec;43(6):673–82.

230. Mazariegos G V, O'Toole K, Mieles LA, Dvorchik I, Meza MP, Briassoulis G, et al. Hyperbaric oxygen therapy for hepatic artery thrombosis after liver

transplantation in children. Liver Transpl Surg. 1999 Sep;5(5):429–36.

231. Grover I, Conley L, Alzate G, Lavine J, Van Hoesen K, Khanna A. Hyperbaric oxygen therapy for hepatic artery thrombosis following liver transplantation: current concepts. Pediatr Transplant. 2006 Mar;10(2):234–9.

232. Ponikvar R, Buturović J, Cizman M, Mekjavić I, Kandus A, Premru V, et al. Hyperbaric oxygenation, plasma exchange, and hemodialysis for treatment of acute liver failure in a 3-year-old child. Artif Organs. 1998 Nov;22(11):952–7.

233. Asanuma Y, Sato T, Yasui O, Kurokawa T, Koyama K. Treatment for postoperative liver failure after major hepatectomy under hepatic total vascular exclusion. J Artif Organs. 2003 Jan;6(2):152–6.

234. Ueno S, Sakoda M, Kurahara H, Iino S, Minami K, Ando K, et al. Safety and efficacy of early postoperative hyperbaric oxygen therapy with restriction of transfusions in patients with HCC who have undergone partial hepatectomy. Langenbeck's Arch Surg / Dtsch Gesellschaft für Chir. 2011 Jan;396(1):99–106.

235. Suehiro T, Shimura T, Okamura K, Okada T, Okada K, Hashimoto S, et al. The effect of hyperbaric oxygen treatment on postoperative morbidity of left lobe donor in living donor adult liver transplantation. Hepatogastroenterology. Jan;55(84):1014–9.

236. Shimizu Y, Miyazaki M, Shimizu H, Ito H, Nakagawa K, Ambiru S, et al. Beneficial effects of arterialization of the portal vein on extended hepatectomy. Br J Surg. 2000 Jun;87(6):784–9.

237. Li J, Cai C, Guo H, Guan X, Yang L, Li Y, et al. Portal vein arterialization promotes liver regeneration after extended partial hepatectomy in a rat model. J Biomed Res. 2015 Jan;29(1):69–75.

238. Chen Y-L, Chen W-B, Wan Y-Y, Li W-G, Huang Z-Q, Wu X-T, et al. Effects of partial portal vein arterialization on liver regeneration after hepatectomy in minipigs with obstructive jaundice. Chin Med J (Engl). 2012 Jul;125(13):2302–5.

239. Bhangui P, Salloum C, Lim C, Andreani P, Ariche A, Adam R, et al. Portal vein arterialization: a salvage procedure for a totally de-arterialized liver. The Paul Brousse Hospital experience. HPB (Oxford). 2014 Aug;16(8):723–38.

240. Qiu J, Chen S, Pankaj P, Wu H. Portal vein arterialization as a bridge procedure against acute liver failure after extended hepatectomy for hilar cholangiocarcinoma. Surg Innov. 2014 Aug;21(4):372–5.

241. Kinoshita H, Sakai K, Hirohashi K, Igawa S, Yamasaki O, Kubo S. Preoperative portal vein embolization for hepatocellular carcinoma. World J Surg. 1986 Oct;10(5):803–8.

242. de Baere T, Denys A, Madoff DC. Preoperative portal vein embolization: indications and technical considerations. Tech Vasc Interv Radiol. 2007 Mar;10(1):67–78.

243. Farges O, Belghiti J, Kianmanesh R, Regimbeau JM, Santoro R, Vilgrain V, et

al. Portal vein embolization before right hepatectomy: prospective clinical trial. Ann Surg. 2003 Feb;237(2):208–17.

244. Ozawa K, Takasan H, Kitamura O, Mizukami T, Kamano T. Effect of ligation of portal vein on liver mitochondrial metabolism. J Biochem. 1971 Nov;70(5):755–64.

245. Katoh T, Tanaka M, Nimura Y, Kanai M, Nagino M, Ozawa T. Enhancement of rat liver mitochondrial function by portal branch ligation secures subsequent extended hepatectomy. Biochem Int. 1991 May;24(1):107–16.

246. Shimizu Y, Suzuki H, Nimura Y, Onoue S, Nagino M, Tanaka M, et al. Elevated mitochondrial gene expression during rat liver regeneration after portal vein ligation. Hepatology. 1995 Oct;22(4 Pt 1):1222–9.

247. Shirasaki K, Taguchi K, Unno M, Motohashi H, Yamamoto M. NF-E2-related factor 2 promotes compensatory liver hypertrophy after portal vein branch ligation in mice. Hepatology. 2014 Jun 25;59(6):2371–82.

248. Adam R, Laurent A, Azoulay D, Castaing D, Bismuth H. Two-stage hepatectomy: A planned strategy to treat irresectable liver tumors. Ann Surg. 2000 Dec;232(6):777–85.

249. Schnitzbauer AA, Lang SA, Goessmann H, Nadalin S, Baumgart J, Farkas SA, et al. Right portal vein ligation combined with in situ splitting induces rapid left lateral liver lobe hypertrophy enabling 2-staged extended right hepatic resection in small-for-size settings. Ann Surg. 2012 Mar;255(3):405–14.

250. Schlegel A, Lesurtel M, Melloul E, Limani P, Tschuor C, Graf R, et al. ALPPS: from human to mice highlighting accelerated and novel mechanisms of liver regeneration. Ann Surg. 2014;260(5):839-46-7.

251. de Santibañes M, Dietrich A, Alvarez FA, Ardiles V, Loresi M, D'adamo 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 Mar;20(3):546–53.

252. Matsuo K, Murakami T, Kawaguchi D, Hiroshima Y, Koda K, Yamazaki 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 Jan 8;159(5):1289–98.

253. Forbes SJ, Gupta S, Dhawan A. Cell therapy for liver disease: From liver transplantation to cell factory. J Hepatol. 2015;62(S1):S157–69.

254. Kuai XL, Cong XQ, Du ZW, Bian YH, Xiao SD. Treatment of surgically induced acute liver failure by transplantation of HNF4-overexpressing embryonic stem cells. Chin J Dig Dis. 2006;7(2):109–16.

255. Cai Y, Zou Z, Liu L, Chen S, Chen Y, Lin Z, et al. Bone marrow-derived mesenchymal stem cells inhibits hepatocyte apoptosis after acute liver injury. Int J Clin Exp Pathol. 2015;8(1):107–16.

256. El'chaninov A V, Volodina MA, Arutyunyan I V, Makarov A V, Tarasova N V, Kananykhina EY, et al. Effect of multipotent stromal cells on the function of cell mitochondria in regenerating liver. Bull Exp Biol Med. 2015 Feb;158(4):566–72.

257. de Andrade DC, de Carvalho SN, Pinheiro D, Thole AA, Moura AS, de Carvalho L, et al. Bone marrow mononuclear cell transplantation improves mitochondrial bioenergetics in the liver of cholestatic rats. Exp Cell Res. Elsevier; 2015 May 12;336(1):15–22.

258. Tautenhahn HM, Bruckner S, Baumann S, Winkler S, Otto W, von Bergen M, et al. Attenuation of Postoperative Acute Liver Failure by Mesenchymal Stem Cell Treatment Due to Metabolic Implications. Ann Surg. 2015;263(3):546–56.

259. Vallabhaneni KC, Haller H, Dumler I. Vascular smooth muscle cells initiate proliferation of mesenchymal stem cells by mitochondrial transfer via tunneling nanotubes. Stem Cells Dev. 2012;21(17):3104–13.

260. Lin H-C, Liu S-Y, Lai H-S, Lai I-R. Isolated mitochondria infusion mitigates ischemia-reperfusion injury of the liver in rats. Shock. 2013;39(3):304–10.

261. Esch JSA, Schmelzle M, Fürst G, Robson SC, Krieg A, Duhme C, et al. Infusion of CD133+ Bone Marrow–Derived Stem Cells After Selective Portal Vein Embolization Enhances Functional Hepatic Reserves After Extended Right Hepatectomy. Ann Surg. 2012;255(1):79–85.

262. Treska V, Liska V, Fichtl J, Lysak D, Mirka H, Bruha J, et al. Portal vein embolisation with application of haematopoietic stem cells in patients with primarily or non-resectable colorectal liver metastases. Anticancer Res. 2014;34(12):7279–85.

263. Leung U, Simpson AL, Araujo RLC, Gönen M, McAuliffe C, Miga MI, et al. Remnant Growth Rate after Portal Vein Embolization Is a Good Early Predictor of Post-Hepatectomy Liver Failure. J Am Coll Surg. 2014 Oct;219(4):620–30.

264. Tralhao JG, Hoti E, Oliveiros B, Botelho MF, Sousa FC. Study of perioperative liver function by dynamic monitoring of ICG-clearance. Hepatogastroenterology. 2012 Jun;59(116):1179–83.

265. Mullin EJ, Metcalfe MS, Maddern GJ. How much liver resection is too much? Am J Surg. 2005 Jul;190(1):87–97.

266. de Santibañes E, Alvarez F a, Ardiles V. How to avoid postoperative liver failure: a novel method. World J Surg. 2012 Jan;36(1):125–8.

267. Shindoh J, Truty MJ, Aloia T a, Curley S a, Zimmitti G, Huang SY, et al. Kinetic growth rate after portal vein embolization predicts posthepatectomy outcomes: toward zero liver-related mortality in patients with colorectal liver metastases and small future liver remnant. J Am Coll Surg. American College of Surgeons; 2013 Feb;216(2):201–9.

268. Wibmer A, Prusa AM, Nolz R, Gruenberger T, Schindl M, Ba-Ssalamah A. Liver failure after major liver resection: risk assessment by using preoperative

Gadoxetic acid-enhanced 3-T MR imaging. Radiology. 2013 Dec;269(3):777–86.

269. Mann D V. Assessment of Liver Function. In: Lau WY, editor. Hilar Cholangiocarcinoma. Springer; 2013. p. 75–89.

270. Palmeira CM, Rolo AP. Mitochondrial membrane potential (ΔΨ) fluctuations associated with the metabolic states of mitochondria. Methods Mol Biol. 2012 Jan;810:89–101.

271. Hall A, Larsen AK, Parhamifar L, Meyle KD, Wu L-P, Moghimi SM. High resolution respirometry analysis of polyethylenimine-mediated mitochondrial energy crisis and cellular stress: Mitochondrial proton leak and inhibition of the electron transport system. Biochim Biophys Acta. 2013 Oct;1827(10):1213–25.

272. Grattagliano I, Caraceni P, Calamita G, Ferri D, Gargano I, Palasciano G, et al. Severe liver steatosis correlates with nitrosative and oxidative stress in rats. Eur J Clin Invest. 2008 Jul;38(7):523–30.

273. Tralhão JG, Hoti E, Oliveiros B, Abrantes AM, Botelho MF, Castro-Sousa F. Intermittent pringle maneuver and hepatic function: Perioperative monitoring by noninvasive ICG-clearance. World J Surg. 2009;33(12):2627–34.

274. Scatton O, Zalinski S, Jegou D, Compagnon P, Lesurtel M, Belghiti J, et al. Randomized clinical trial of ischaemic preconditioning in major liver resection with intermittent Pringle manoeuvre. Br J Surg. 2011;98:1236–43.

275. Martins PN a, Theruvath TP, Neuhaus P. Rodent models of partial hepatectomies. Liver Int. 2008 Jan;28(1):3–11.

276. Palmeira CM, Moreno AJ, Madeira VM. Interactions of herbicides 2,4-D and dinoseb with liver mitochondrial bioenergetics. Toxicol Appl Pharmacol. 1994 Jul;127(1):50–7.

277. Chance B, Williams GR. Respiratory enzymes in oxidative phosphorylation. VI. The effects of adenosine diphosphate on azide-treated mitochondria. J Biol Chem. 1956 Jul;221(1):477–89.

278. Stocchi V, Cucchiarini L, Magnani M, Chiarantini L, Palma P, Crescentini G. Simultaneous extraction and reverse-phase high-performance liquid chromatographic determination of adenine and pyridine nucleotides in human red blood cells. Anal Biochem. 1985 Apr;146(1):118–24.

279. Wu J-F, Wu R-Y, Chen J, Ou-Yang B, Chen M-Y, Guan X-D. Early lactate clearance as a reliable predictor of initial poor graft function after orthotopic liver transplantation. Hepatobiliary Pancreat Dis Int. 2011 Dec;10(6):587–92.

280. Dindo D, Demartines N, Clavien P. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. Ann Surg. 2004 Aug;240(2):205–13.

281. Koch M, Garden OJ, Padbury R, Rahbari NN, Adam R, Capussotti L, et al. Bile leakage after hepatobiliary and pancreatic surgery: A definition and grading of severity by the International Study Group of Liver Surgery. Surgery.

2011;149(5):680–8.

282. Mann CD, Palser T, Briggs CD, Cameron I, Rees M, Buckles J, et al. A review of factors predicting perioperative death and early outcome in hepatopancreaticobiliary cancer surgery. Hepatobiliary Pancreat Dis Int. 2010;12(6):380–8.

283. Ypsilantis P, Lambropoulou M, Tentes I, Anagnostopoulos K, Tsigalou C, Papadopoulos N, et al. Impaired liver regeneration following partial hepatectomy using the Pringle maneuver: Protective effect of mesna. J Gastroenterol Hepatol. 2009 Apr;24(4):623–32.

284. Seyama Y, Imamura H, Inagaki Y, Matsuyama Y, Tang W, Makuuchi M, et al. Intermittent clamping is superior to ischemic preconditioning and its effect is more marked with shorter clamping cycles in the rat liver. J Gastroenterol. 2013 Jan;48(1):115–24.

285. van der Bilt JDW, Livestro DP, Borren a, van Hillegersberg R, Borel Rinkes IHM. European survey on the application of vascular clamping in liver surgery. Dig Surg. 2007 Jan;24(6):423–35.

286. Rudnick DA, Davidson NO. Functional Relationships between Lipid Metabolism and Liver Regeneration. Int J Hepatol. 2012;10(15).

287. Benzoni E, Lorenzin D, Baccarani U, Adani GL, Favero A, Cojutti A, et al. Resective surgery for liver tumor: a multivariate analysis of causes and risk factors linked to postoperative complications. Hepatobiliary Pancreat Dis Int. 2006 Nov;5(4):526–33.

288. Boleslawski E, Vibert E, Pruvot F-R, Le Treut Y-P, Scatton O, Laurent C, et al. Relevance of postoperative peak transaminase after elective hepatectomy. Ann Surg. 2014 Nov;260(5):815-20-1.

289. Lafaro K, Buettner S, Maqsood H, Wagner D, Bagante F, Spolverato G, et al. Defining Post Hepatectomy Liver Insufficiency: Where do We stand? J Gastrointest Surg. 2015 Nov;19(11):2079–92.

290. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JWW, Comber H, et al. Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. Eur J Cancer. 2013 Apr;49(6):1374–403.

291. Fong Y, Fortner J, Sun RL, Brennan MF, Blumgart LH. Clinical Score for Predicting Recurrence After Hepatic Resection for Metastatic Colorectal Cancer Analysis of 1001 Consecutive Cases. 1999;230(3):309–21.

292. Kopetz S, Chang GJ, Overman MJ, Eng C, Sargent DJ, Larson DW, et al. Improved survival in metastatic colorectal cancer is associated with adoption of hepatic resection and improved chemotherapy. J Clin Oncol. 2009;27(22):3677–83.

293. Blazer DG, Kishi Y, Maru DM, Kopetz S, Chun YS, Overman MJ, et al. Pathologic response to preoperative chemotherapy: a new outcome end point

after resection of hepatic colorectal metastases. J Clin Oncol. 2008 Nov;26(33):5344–51.

294. Kishi Y, Zorzi D, Contreras CM, Maru DM, Kopetz S, Ribero D, et al. Extended preoperative chemotherapy does not improve pathologic response and increases postoperative liver insufficiency after hepatic resection for colorectal liver metastases. Ann Surg Oncol. 2010;17(11):2870–6.

295. Rubbia-Brandt L, Audard V, Sartoretti P, Roth AD, Brezault C, Le Charpentier M, et al. Severe hepatic sinusoidal obstruction associated with oxaliplatin-based chemotherapy in patients with metastatic colorectal cancer. Ann Oncol. 2004 Mar;15(3):460–6.

296. Hubert C, Sempoux C, Humblet Y, Van Den Eynde M, Zech F, Leclercq I, et al. Sinusoidal obstruction syndrome (SOS) related to chemotherapy for colorectal liver metastases: Factors predictive of severe SOS lesions and protective effect of bevacizumab. HPB. 2013;15:858–64.

297. Soubrane O, Brouquet A, Zalinski S, Terris B, Brézault C, Mallet V, et al. Predicting High Grade Lesions of Sinusoidal Obstruction Syndrome Related to Oxaliplatin-Based Chemotherapy for Colorectal Liver Metastases. Ann Surg. 2010 Mar;251(3):454–60.

298. Alexandrino H, Oliveira D, Cipriano MA, Ferreira L, Tralhão JG, Castro E Sousa F. Oxaliplatin toxicity presenting as a liver nodule - case report. BMC Cancer. 2015 Jan;15:247.

299. Uchino K, Fujisawa M, Watanabe T, Endo Y, Nobuhisa T, Matsumoto Y, et al. Oxaliplatin-induced liver injury mimicking metastatic tumor on images: a case report. Jpn J Clin Oncol. 2013 Oct;43(10):1034–8.

300. Xiong W-J, Hu L-J, Jian Y-C, He Y, Zhou W, Guo X-L, et al. Focal peliosis hepatis in a colon cancer patient resembling metastatic liver tumor. World J Gastroenterol. 2012 Nov 7;18(41):5999–6002.

301. Choi J-H, Won Y-W, Kim HS, Oh Y-H, Lim S, Kim H-J. Oxaliplatin-induced sinusoidal obstruction syndrome mimicking metastatic colon cancer in the liver. Oncol Lett. 2016 Apr;11(4):2861–4.

302. Kawai T, Yamazaki S, Iwama A, Higaki T, Sugitani M, Takayama T. Focal Sinusoidal Obstruction Syndrome Caused by Oxaliplatin-Induced Chemotherapy: A Case Report. Hepat Mon. 2016 Aug 21;16(9):e37572.

303. Overman MJ, Maru DM, Charnsangavej C, Loyer EM, Wang H, Pathak P, et al. Oxaliplatin-Mediated Increase in Spleen Size As a Biomarker for the Development of Hepatic Sinusoidal Injury. J Clin Oncol. 2010;28(15):2549–55.

304. Shin N-Y, Kim M-J, Lim JS, Park M-S, Chung Y-E, Choi J-Y, et al. Accuracy of gadoxetic acid-enhanced magnetic resonance imaging for the diagnosis of sinusoidal obstruction syndrome in patients with chemotherapy-treated colorectal liver metastases. Eur Radiol. 2012 Apr;22(4):864–71.

305. Wibmer A, Prusa AM, Nolz R, Gruenberger T, Schindl M, Ba-Ssalamah A. Liver failure after major liver resection: risk assessment by using preoperative Gadoxetic acid-enhanced 3-T MR imaging. Radiology. 2013 Dec;269(3):777–86.

306. van Mierlo KMC, Zhao J, Kleijnen J, Rensen SS, Schaap FG, Dejong CHC, et al. The influence of chemotherapy-associated sinusoidal dilatation on short-term outcome after partial hepatectomy for colorectal liver metastases: A systematic review with meta-analysis. Surg Oncol. 2016 Sep;25(3):298–307.

307. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005 Jun;41(6):1313–21.

308. Boeykens N, Ponsaerts P, Ysebaert D, Greef K De. Biochemical Parameters for Longitudinal Monitoring of Liver Function in Rat Models of Partial Hepatectomy Following Liver Injury. PLoS One. 2013;8(6):2–9.

309. Zhou S, Palmeira CM, Wallace KB. Doxorubicin-induced persistent oxidative stress to cardiac myocytes. Toxicol Lett. 2001 May 19;121(3):151–7.

310. Palmeira CM, Wallace KB. Benzoquinone inhibits the voltage-dependent induction of the mitochondrial permeability transition caused by redox-cycling naphthoquinones. Toxicol Appl Pharmacol. 1997 Apr;143(2):338–47.

311. Vreuls CPH, Driessen A, Olde Damink SWM, Koek GH, Duimel H, van den Broek MAJ, et al. Sinusoidal obstruction syndrome (SOS): A light and electron microscopy study in human liver. Micron. 2016 May;84:17–22.

312. Reissfelder C, Brand K, Sobiegalla J, Rahbari NN, Bork U, Schirmacher P, et al. Chemotherapy-associated liver injury and its influence on outcome after resection of colorectal liver metastases. Surgery. 2014 Feb;155(2):245–54.

313. Viganò L, Capussotti L, De Rosa G, De Saussure WO, Mentha G, Rubbia-Brandt L. Liver resection for colorectal metastases after chemotherapy: impact of chemotherapy-related liver injuries, pathological tumor response, and micrometastases on long-term survival. Ann Surg. 2013;258(5):731-40-2.

314. Pilgrim CHC, Satgunaseelan L, Pham A, Murray W, Link E, Smith M, et al. Correlations between histopathological diagnosis of chemotherapy-induced hepatic injury, clinical features, and perioperative morbidity. 2012;15(5):333–40.

315. Tamandl D, Klinger M, Eipeldauer S, Herberger B, Kaczirek K, Gruenberger B, et al. Sinusoidal Obstruction Syndrome Impairs Long-Term Outcome of Colorectal Liver Metastases Treated with Resection after Neoadjuvant Chemotherapy. Ann Surg Oncol. 2011;18(2):421–30.

316. Vreuls CPH, Olde Damink SWM, Koek GH, Winstanley A, Wisse E, Cloots RHE, et al. Glutathione S-transferase M1-null genotype as risk factor for SOS in oxaliplatin-treated patients with metastatic colorectal cancer. Br J Cancer. Nature Publishing Group; 2013;108(January):1–5.

317. Huezo-Diaz Curtis P, Uppugunduri CRS, Muthukumaran J, Rezgui MA, Peters

C, Bader P, et al. Association of CTH variant with sinusoidal obstruction syndrome in children receiving intravenous busulfan and cyclophosphamide before hematopoietic stem cell transplantation. Pharmacogenomics J. 2016 Oct 25;(doi: 10.1038/tpj.2016.65. [Epub ahead of print]).

318. Brouquet A, Benoist S, Julie C, Penna C, Beauchet A, Rougier P, et al. Risk factors for chemotherapy-associated liver injuries: A multivariate analysis of a group of 146 patients with colorectal metastases. Surgery. 2009;145(4):362–71.

319. Rubbia-Brandt L, Tauzin S, Brezault C, Delucinge-Vivier C, Descombes P, Dousset B, et al. Gene expression profiling provides insights into pathways of oxaliplatin-related sinusoidal obstruction syndrome in humans. Mol Cancer Ther. 2011 Apr;10(4):687–96.

320. Agostini J, Benoist S, Seman M, Julié C, Imbeaud S, Letourneur F, et al. Identification of molecular pathways involved in oxaliplatin-associated sinusoidal dilatation. J Hepatol. European Association for the Study of the Liver; 2012;56(4):869–76.

321. DeLeve LD, McCuskey RS, Wang X, Hu L, McCuskey MK, Epstein RB, et al. Characterization of a reproducible rat model of hepatic veno-occlusive disease. Hepatology. 1999 Jun;29(6):1779–91.

322. Jafari A, Wehner S, Kalff JC, Manekeller S. Sinusoidal obstruction syndrome in the animal model: influence on liver surgery. Langenbeck's Arch Surg. 2016 Sep 1;[Epub ahead of print].

323. Rickenbacher A, DeOliveira ML, Tian Y, Jang JH, Riener M-O, Graf R, et al. Arguments against toxic effects of chemotherapy on liver injury and regeneration in an experimental model of partial hepatectomy. Liver Int. 2011 Mar;31(3):313–21.

324. Hubert C, Dahrenmoller C, Marique L, Jabbour N, Gianello P, Leclercq I. Hepatic regeneration in a rat model is impaired by chemotherapy agents used in metastatic colorectal cancer. Eur J Surg Oncol. 2015 Aug 21;41(11):1471–8.

325. Robinson SM, Mann J, Manas DM, Mann DA, White SA. An experimental study to identify the potential role of pharmacogenomics in determining the occurrence of oxaliplatin-induced liver injury. HPB. 2013;15(8):581–7.

326. Katano K, Kondo A, Safaei R, Holzer A, Samimi G, Mishima M, et al. Acquisition of resistance to cisplatin is accompanied by changes in the cellular pharmacology of copper. Cancer Res. 2002 Nov 15;62(22):6559–65.

327. Lichtmannegger J, Leitzinger C, Wimmer R, Schmitt S, Schulz S, Kabiri Y, et al. Methanobactin reverses acute liver failure in a rat model of Wilson disease. J Clin Invest. 2016 Jun 20;126(7):2721–35.

328. Kim H, Baek S-E, Mo--on J, Roh YH, Lee N, Cho A. Increased hepatic FDG uptake on PET/CT in hepatic sinusoidal obstructive syndrome. Oncotarget. 2015 Jul 18;7(42):69024–69031.

329. Liemburg-Apers DC, Schirris TJJ, Russel FGM, Willems PHGM, Koopman WJH. Mitoenergetic Dysfunction Triggers a Rapid Compensatory Increase in Steady-State Glucose Flux. Biophys J. 2015 Oct;109(7):1372–86.

330. DeLeve L. Hepatic Microvasculature in Liver Injury. Semin Liver Dis. 2007 Nov;27(4):390–400.

331. Narita M, Oussoultzoglou E, Chenard M-P, Fuchshuber P, Rather M, Rosso E, et al. Liver Injury Due to Chemotherapy-induced Sinusoidal Obstruction Syndrome Is Associated with Sinusoidal Capillarization. Ann Surg Oncol. 2012 Jul 9;19(7):2230–7.

332. Duarte FV, Gomes AP, Teodoro JS, Varela AT, Moreno AJM, Rolo AP, et al. Dibenzofuran-induced mitochondrial dysfunction: Interaction with ANT carrier. Toxicol Vitr. 2013 Dec;27(8):2160–8.

333. Li Y, Couch L, Higuchi M, Fang J-L, Guo L. Mitochondrial Dysfunction Induced by Sertraline, an Antidepressant Agent. Toxicol Sci. 2012 Jun 1;127(2):582–91.

334. Lentschener C, Nicco C, Terris B, Samama C-M, Coriat R. Comment on "The potential contribution of tumour-related factors to the development of FOLFOX-induced sinusoidal obstruction syndrome." Br J Cancer. 2016 Oct 11;115(8):e7.

335. Chua W, Kho PS, Moore MM, Charles KA, Clarke SJ. Clinical, laboratory and molecular factors predicting chemotherapy efficacy and toxicity in colorectal cancer. Crit Rev Oncol / Hematol. Elsevier Ireland Ltd; 2011;79(3):224–50.

336. Bale SS, Golberg I, Jindal R, McCarty WJ, Luitje M, Hegde M, et al. Long-term coculture strategies for primary hepatocytes and liver sinusoidal endothelial cells. Tissue Eng Part C Methods. 2015 Apr;21(4):413–22.

337. Bale SS, Geerts S, Jindal R, Yarmush ML. Isolation and co-culture of rat parenchymal and non-parenchymal liver cells to evaluate cellular interactions and response. Sci Rep. 2016 May 4;6:25329.

338. Schadde E, Ardiles V, Robles-Campos R, Malago M, Machado M, Hernandez-Alejandro R, et al. Early survival and safety of ALPPS: first report of the International ALPPS Registry. Ann Surg. 2014 Nov;260(5):829-36-8.

339. Eshmuminov D, Raptis DA, Linecker M, Wirsching A, Lesurtel M, Clavien P-A. Meta-analysis of associating liver partition with portal vein ligation and portal vein occlusion for two-stage hepatectomy. Br J Surg. 2016 Sep;103(13):1768–82.

340. de Santibañes E, Alvarez FA, Ardiles V, Pekolj J, de Santibañes M. Inverting the ALPPS paradigm by minimizing first stage impact: the Mini-ALPPS technique. Langenbeck's Arch Surg / Dtsch Gesellschaft für Chir. 2016 Jun;401(4):557–63.

341. Linecker M, Kambakamba P, Reiner CS, Linh Nguyen-Kim TD, Stavrou GA, Jenner RM, et al. How much liver needs to be transected in ALPPS? A translational study investigating the concept of less invasiveness. Surgery. 2016 Nov 1;161(2):453–64.

342. Serenari M, Collaud C, Alvarez FA, de Santibañes M, Giunta D, Pekolj J, et al.

Interstage Assessment of Remnant Liver Function in ALPPS Using Hepatobiliary Scintigraphy: Prediction of Posthepatectomy Liver Failure and Introduction of the HIBA Index. Ann Surg. 2017 Jan 24;Jan 24(doi: 10.1097/SLA.0000000000002150. [Epub ahead of print]).

343. Kambakamba P, Stocker D, Reiner CS, Nguyen-Kim TD, Linecker M, Eshmuminov D, et al. Liver kinetic growth rate predicts postoperative liver failure after ALPPS. HPB (Oxford). 2016 Aug 11;18(10):800–5.

344. Vauthey J-N, Abdalla EK, Doherty DA, Gertsch P, Fenstermacher MJ, Loyer EM, et al. Body surface area and body weight predict total liver volume in Western adults. Liver Transpl. 2002 Mar;8(3):233–40.

345. Mosteller RD. Simplified calculation of body-surface area. N Engl J Med. 1987 Oct 22;317(17):1098.

346. Schadde E, Malagó M, Hernandez-Alejandro R, Li J, Abdalla E, Ardiles V, et al. Monosegment ALPPS hepatectomy: Extending resectability by rapid hypertrophy. Surgery. 2015 Apr;157(4):676–89.

347. Belghiti J, Dokmak S, Schadde E. ALPPS: Innovation for innovation's sake. Surgery. 2016 May;159(5):1287–8.

348. Komori K, Nagino M, Nimura Y. Hepatocyte morphology and kinetics after portal vein embolization. Br J Surg. 2006 Jul;93(6):745–51.

349. van de Laarschot LFM, Jansen PLM, Schaap FG, Olde Damink SWM. The role of bile salts in liver regeneration. Hepatol Int. 2016 Sep 5;10(5):733–40.

350. Valdecantos MP, Pardo V, Ruiz L, Castro-Sánchez L, Lanzón B, Fernández-Millán E, et al. A novel glucagon-like peptide 1/glucagon receptor dual agonist improves steatohepatitis and liver regeneration in mice. Hepatology. 2016 Nov 23;doi: 10.1002/hep.28962. [Epub ahead of print].

351. Ding L, Yang Y, Qu Y, Yang T, Wang K, Liu W, et al. Bile acid promotes liver regeneration via farnesoid X receptor signaling pathways in rats. Mol Med Rep. 2015 Jan 29;11(6):4431–7.

352. Zeile M, Bakal A, Volkmer JE, Stavrou GA, Dautel P, Hoeltje J, et al. Identification of cofactors influencing hypertrophy of the future liver remnant after portal vein embolization-the effect of collaterals on embolized liver volume. Br J Radiol. 2016 Dec;89(1068):20160306.

353. Olthof PB, Schadde E, van Lienden KP, Heger M, de Bruin K, Verheij J, et al. Hepatic parenchymal transection increases liver volume but not function after portal vein embolization in rabbits. Surgery. 2017 Feb 4;(doi: 10.1016/j.surg.2016.12.014. [Epub ahead of print]).

354. Jin X, Zhang Z, Beer-Stolz D, Zimmers TA, Koniaris LG. Interleukin-6 inhibits oxidative injury and necrosis after extreme liver resection. Hepatology. 2007 Sep;46(3):802–12.

355. Herminghaus A, Barthel F, Heinen A, Beck C, Vollmer C, Bauer I, et al. Severity

of polymicrobial sepsis modulates mitochondrial function in rat liver. Mitochondrion. 2015 Sep;24:122–8.

356. Yoon JH, Choi J-I, Jeong YY, Schenk A, Chen L, Laue H, et al. Pre-treatment estimation of future remnant liver function using gadoxetic acid MRI in patients with HCC. J Hepatol. 2016 Dec;65(6):1155–62.

357. de Santibañes E, Clavien P-A. Playing Play-Doh to Prevent Postoperative Liver Failure. Ann Surg. 2012 Mar;255(3):415–7.

358. Guiu B, Chevallier P, Denys A, Delhom E, Pierredon-Foulongne M-A, Rouanet P, et al. Simultaneous trans-hepatic portal and hepatic vein embolization before major hepatectomy: the liver venous deprivation technique. Eur Radiol. 2016 Dec 18;26(12):4259–67.

359. Mortensen KE, Conley LN, Nygaard I, Sorenesen P, Mortensen E, Bendixen C, et al. Increased sinusoidal flow is not the primary stimulus to liver regeneration. Comp Hepatol. 2010 Jan 20;9(1):2.

360. Hohmann N, Weiwei W, Dahmen U, Dirsch O, Deutsch A, Voss-Böhme A. How Does a Single Cell Know When the Liver Has Reached Its Correct Size? Avila MA, editor. PLoS One. 2014 Apr 1;9(4):e93207.

361. Michalopoulos GK. Hepatostat: Liver regeneration and normal liver tissue maintenance. Hepatology. 2016 Dec 20;(doi: 10.1002/hep.28988. [Epub ahead of print]).