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Staufenberg, am 18.06.2021

Eidesstattliche Erklärung

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Abbreviations

ACH	acetylcholine
ADAMTS-13	a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13
ADH	alcohol dehydrogenase
ADP	adenosine diphosphate
ALD	acute liver disease
ALDH	aldehyde dehydrogenase
ANCA	anti-neutrophil cytoplasmic antibody
aPTT	activated partial thrombin time
ASH	alcoholic steatohepatitis
AT(III)	antithrombin (III)
ATP	adenosine triphosphate
bFGF	basic fibroblast growth factor
Ca2+	calcium
cAMP	cyclic adenosine monophosphate
CCC	cholangiocellular carcinoma
CCT	conventional coagulation tests
CFT	clot formation time
cGMP	cyclic guanosine monophosphate
CLD	chronic liver disease
CMV	cytomegalovirus
CO_2	carbon dioxide
CRC	colorectal carcinoma
CT	clotting time
CVS	cardiovascular system
DVT	deep vein thrombosis
EBV	Epstein-Barr virus
EDRF	endothelium-derived relaxing factor
eNOS	endothelial nitric oxide synthetase
ET-1	endothelin 1
ETP	endogenous thrombin potential
FFP	fresh frozen plasma
FNH	focal nodular hyperplasia

GC	guanylate cyclase
GP	glycoprotein
GSH	glutathione
GTP	guanosine triphosphate
HCC	hepatocellular carcinoma
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HMW	high molecular weight
HMWK	high molecular weight kinogen
HSC	hepatic stellate cell
IL-1	interleukin 1
IDL	intermediate-density lipoprotein
iNOS	inducible nitric oxide synthase
INR	international normalized ratio
L-ARG	L-arginine
LDL	low density lipoprotein
LI 30	Lysis after 30 minutes
MEOS	microsomal ethanol oxidizing system
MCF	maximum clot firmness
ML	maximum lysis
MI	myocardial ischemia
NADH/ NAD+	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
NAFLD	non-alcoholic fatty liver disease
NAFL	non-alcoholic fatty liver
NASH	non-alcoholic steatohepatitis
nNO	neuronal nitric oxide synthase
NO	nitric oxide
NOS	nitric oxide synthase
NSAID	non-steroidal anti-inflammatory drugs
O_2	oxygen
PAF	platelet activating factor
PAI-1	plasminogen activator inhibitor-1
PAN	polyarteritis nodosa

PC	platelet count
PDGF	platelet derived growth factor
PE	pulmonary embolism
PFA	platelet function assay
PGI_2	prostaglandin I2, prostacyclin
POD	postoperative day
PPPD	pylorus-preserving pancreaticoduodenectomy
PT	prothrombin time
ROTEM®	rotational thromboelastometry
rtPA	recombinant tissue plasminogen activator
SMC	smooth muscle cell
TAFI	thrombin activatable fibrinolysis inhibitor
TAG	triacylglycerol
TEG®	thromboelastography
TF	tissue factor
TG	thrombin generation
TGA	thrombin generation assay
TGF-ß	transforming growth factor ß
TM	thrombomodulin
TM-SR	thrombomodulin sensitivity ratio
tPA	tissue plasminogen activator
TT	thrombin time
TxA2	thromboxane A2
VEGF	vascular endothelial growth factor
VET	viscoelastic tests
VLDL	very low density lipoprotein
VTE	venous thromboembolism
vWF	von Willebrand factor
WHO	World Health Organization

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Zusammenfassung

Einleitung: Lebererkrankungen kommen häufig in der Gesellschaft vor. Die meisten leberchirurgischen Eingriffe werden aufgrund von benignen oder malignen Leberläsionen durchgeführt. In der Synthese und im Abbau der Proteine der Blutgerinnung und der Fibrinolyse, spielt die Leber die wohl wichtigste Rolle. Daher lässt sich ein Einfluss von Verlust an Leberparenchym und -funktion nach leberchirurgischen Eingriffen auf die Hämostase vermuten. Veränderungen der Hämostase können die Gefäßgesundheit gefährden, indem sie einerseits das Risiko für Blutungen oder andererseits das für Blutgerinnsel erhöhen. Thrombosen und Thromboembolien können zu Gefäßverschlüssen und in Folge zu Gefäß- sowie Organdysfunktion und -schäden führen. Die weltweit häufigsten Todesursachen, ischämische Herzerkrankung und Schlaganfall, werden in den meisten Fällen durch einen thromboembolischen Gefäßverschluss oder Thromben aufgrund rupturierter atherosklerotischer Plaques verursacht. Durch die Identifizierung der Einflüsse von leberchirurgischen Eingriffen auf die Blutgerinnung kann eine adäquate Therapie etabliert und damit die Gefäßgesundheit geschützt werden.

Das Ziel dieser Diplomarbeit war die Bereitstellung einer Übersicht der aktuellen Studien über den Effekt einer Hepatektomie auf die Blutgerinnung, als ein möglicher Aspekt des Einflusses von leberchirurgischen Eingriffen auf die Gefäßgesundheit.

Methoden: Die systemische Literaturrecherche erfasste Publikationen zum Effekt einer Hepatektomie auf die Blutgerinnung. PubMed und Web of Science wurden dabei als Suchmaschinen genutzt. Der Zugang zu den meisten Publikationen konnte durch die Medizinische Universität Graz gewährleistet werden. Es wurden Studien herangezogen, die seit 2015 veröffentlicht wurden und im perioperativen Setting einer Hepatektomie Parameter der Blutgerinnung oder Fibrinolyse bestimmt haben. Die als relevant definierten Studien wurden weiter in zwei Gruppen eingeteilt: 1) Kurative Hepatektomie bei Lebererkrankungen und 2) Hepatektomie in lebergesunden Lebendorganspendern.

Ergebnisse/: Die initiale Literaturrecherche ergab 2862 Titel, von denen 15 Publikationen zur näheren Auswertung und Analyse herangezogen wurden.

Diskussion/Schlussfolgerung: Nach Leberresektionen (Hepatektomie) konnte durch Thrombin-Generation-Assay oder durch viskoelastische Tests entweder

eine normale Blutgerinnung oder eine vorübergehende Hyperkoagulabilität gezeigt werden, während die konventionellen Gerinnungstest eine verminderte Blutgerinnung vermuten ließen. Die viskoelastischen Tests können Ergebnisse gewährleisten, welche der Gerinnung in vivo näherkommen. Durch zusätzliche Messungen einzelner Gerinnungsfaktoren im perioperativen Setting, konnte gezeigt werden, dass Leberresektionen bei Lebererkrankten einen potenziell prothrombotischen Zustand begünstigen. In Hinsicht auf ein erhöhtes Risiko für Thromboembolien, wird die Gefäßgesundheit durch den leberchirurgischen Eingriff gefährdet.

Abstract

Introduction: Liver diseases are common in today's society. The main reasons for liver surgery are benign and malignant lesions, with some disease stages even requiring liver transplantation. The liver is the most important organ in terms of synthesis and clearance of proteins responsible for hemostasis. With impaired liver function and loss of parenchyma after liver resection, hemostasis is assumingly affected. Disturbances in hemostasis affect vascular health by either promoting bleeding or blood clotting. Thrombosis and thromboembolism can lead to vascular occlusion and following vascular and organ dysfunction. Vascular occlusion which leads to ischemic heart disease and stroke, the two leading causes of death worldwide, is in most cases caused by thrombi due to ruptured atherosclerotic plaques or by thromboembolism. Identifying the effects of liver surgery on hemostasis is crucial for determining adequate treatment and ensuring vascular health.

Aims and objectives: The aim of this thesis is to present an overview of recent study results on the effects of hepatectomy on hemostasis, as one aspect of liver surgery affecting vascular health.

Methodology: A systemic research of the currently published literature on the effects of hepatectomy on hemostasis was conducted. Search engines used were PubMed and Web of Science. Access to most publications was given through the Medical University of Graz. Studies published in 2015 and following years, in which parameters of hemostasis or fibrinolysis were measured perioperative to hepatectomy were considered. Relevant studies were further grouped into curative hepatectomy in liver disease and hepatectomy for living organ donation.

Results: The literature research resulted in 2862 publications, of which 15 publications met all inclusion criteria.

Discussion: Following liver resection, measurements suggested different states of hemostasis. While thrombin generation assay and viscoelastic tests identified either normal hemostasis or a transient hypercoagulable state, conventional coagulation parameters suggested a hypocoagulable state. With viscoelastic tests providing a more realistic picture of hemostasis in vivo, and in addition to individual hemostatic parameter levels measured perioperatively, curative hepatectomy was shown to promote a potentially prothrombotic state. Therefore, vascular health is affected by liver surgery in terms of an increased risk for thromboembolism.

1 Introduction

To gain an understanding of how liver surgery can affect vascular health, first, an overview of the vascular system is provided. As it is a crucial component of vascular heath, a special focus lays on hemostasis. Followed by that, on overview of liver function, diseases, surgery, and its role in hemostasis is given.

1.1 The Vascular System

The cardiovascular system (CVS) consists of the blood vessels (arterial, venous and capillary system), the heart and the blood. Including the lymphatic system, one refers to the vascular system (or circulatory system) (1) (2). It is one of the largest and most vulnerable organ systems of the human body. It fulfills different tasks, as e.g. transporting oxygen (O_2) and nutrients to the tissues in exchange to carbon dioxide (CO_2) and “waste products”/metabolites, enabling intercellular communication by transporting hormones, regulating blood pressure, body temperature and pH and, by this all, maintaining homeostasis (1) (2). A well-functioning vascular system is therefore crucial for all vital functions (2). In the following, the focus lays on the blood vessels.

1.1.1 The Blood Vessels

When referring to blood vessels, there are mainly three types being differentiated: arteries, veins and capillaries. The specific characteristics are described in the following chapters. All types of blood vessel walls, with exceptions of the microcirculatory system, consist of three microscopic layers (from internal to external): tunica intima, tunica media and tunica adventitia (3), as seen in Figure 1.

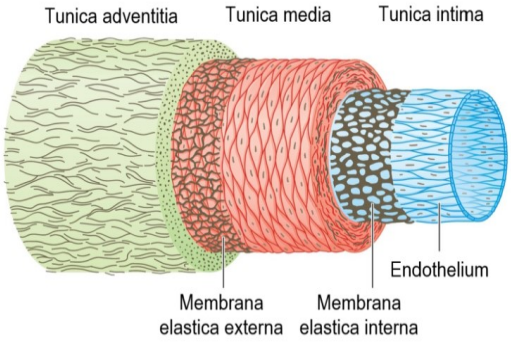

Figure 1: Arterial vessel wall, muscular type (modified after (3))

Within the tunica intima three layers can be differentiated: the endothelium, the subendothelial layer, and the internal elastic lamina. The tunica media consists of smooth muscle cells, as well as elastic and collagenous fibers. Within the tunica media, smooth muscle cells are dominating, enabling muscular regulation of the vessels´ lumen, and by that also changing the resistance. The tunica adventitia consists of connecting tissue, which integrates vasa vasorum, nerves, and lymphatic vessels (3). In veins, the three layers show a greater variability and are less distinct (3) (1) (4). In contrast to the described structure of arteries and veins, the capillary´s wall consists only of the endothelium and a basal layer with pericytes on its outside. The capillary´s endothelium is categorized by its histological appearance (3). The most common type is continuous endothelium, which is also found in most other blood vessels, showing a low permeability. Another type is fenestrated endothelium, as found in glomerular capillaries. It shows clefts between endothelial cells but a continuous basal membrane. Discontinuous endothelium, which is further grouped into perforated endothelium, as found in liver sinusoids and in bone marrow, and disjunct endothelium, as found in venous sinusoids of the spleen, shows gaps between endothelial cells and the basal membrane (5) (3).

1.1.1.1 The Arterial System

The arterial system transports oxygenated blood from the heart to the periphery (2). (With exception of the pulmonary artery and the umbilical arteries in the fetus) (1). It can be differentiated by size into large (arteries) and small vessels (arterioles, metarterioles). Depending on which component is dominating in the arterial wall´s tunica media, an artery is classified as either elastic or muscular type. The elastic type is seen in the large arteries close to the heart (Aorta, Truncus pulmonalis, and branches) (3). A great amount of elastic membranes enables the aorta to stretch during systole and by this to store blood. During diastole, the aorta goes back into its non-stretched status and by this moving the stored blood forward. This effect is called “windkessel effect” and secures a constant blood flow within the arteries (2) (1). The muscular type is found in arteries located farer away from the heart (e.g. A. brachialis, A. femoralis, arterioles) (3). The arterioles, the smallest arteries in the human body, show a high percentage of smooth muscle within the vessel wall. They make up the greatest

percentage of the peripheral resistance and are therefore also referred to as “resistance vessels” (2). The ability of muscular regulation of the lumen, regulating vascular resistance, is crucial of adapting the blood flow to changed needs of the body. There are several factors leading to either dilated (vasodilation) or constricted (vasoconstriction) vessels. Within the arterial system there is a high internal pressure, ensuring organ perfusion and requiring the thick wall of arteries (6).

1.1.1.2 The Venous System

The venous system persists of blood vessels coming from the periphery/organs/tissues back to the heart. Blood from the capillaries flows into post-capillary venules, the smallest veins within the human body. As the endothelial layer is not very tight, post-capillary venules show a high permeability (3). From the venules, blood flows into the larger veins and back to the right heart. With exception of the pulmonary veins, venous blood is low in oxygen. Within the venous system, blood pressure is much lower than in the arterial system, hence less elastic tissue and a much thinner vessel wall (1). In contrast to the arterial system, in which a small amount of the total blood volume circulates with a high pressure, in the venous system almost 85% of blood volume circulate with a comparable low pressure (2). This gives the venous system also the label “capacity system” (2). For blood to flow towards the heart, even under low pressure and against gravity, valves can be found in many veins, especially in the extremities. These inhibit the blood in veins to flow in retrograde direction (3).

1.1.2 Vascular Function

While it would exceed the purpose of this work to describe all mechanisms the vascular system maintains its homeostasis by, under physiological and pathophysiological conditions, it can be said that there are multiplex processes involved. Adapting to changing needs, a well-functioning, healthy vascular system is crucial. In the following the focus lays on the endothelium as the inner lining of almost all vessels and involved in most processes ensuring vascular homeostasis.

1.1.2.1 The Endothelium

The endothelium, as part of the tunica intima, lines the inner surface of blood and lymphatic vessels. Taken the endothelial surfaces of the capillaries and the venules together, a surface of approximately 1000m² is reached (2). The endothelium functions as a diffusion barrier of macromolecules into the subendothelial layer, while selective permeability is possible. There are different structures of the endothelium seen in different locations within the human body: Continuous endothelium as in the central nervous system, fenestrated endothelium as in the gastrointestinal tract or discontinuous endothelium as in liver sinusoids (3). The endothelium, being remarkably metabolic active and in permanent dynamic response to changing needs, plays an essential role in both regulating physiological as well as pathophysiological process. (7)

Vessel width and tone

Vasodilation and vasoconstriction is pivotal in regulation of organ perfusion. While it is the muscular layer of the tunica media that causes wall tension, and therefore regulating vasodilation and vasoconstriction, it is regulated in this by molecular mediators secreted from the endothelium. The role of the endothelium in relaxation of a vessel´s smooth muscles was described in 1980 by Furchgott, who named the mediator “endothelium-derived relaxing factor (EDRF)” (8). The mediator was identified as nitric oxide (NO) (9).

NO is a small endogenously produced radical. Since NO is a gas, it is capable to pass cell membranes without further transporting systems. It is synthesized from the amino acid L-arginine (L-ARG) by NO-synthases (NOS). There are three isoforms of NOS: neuronal NOS (nNOS), inducible NOS (iNOS) and endothelial NOS (eNOS). While nNOS and eNOS are dependent on calcium and constitutively produced, iNOS is not. Acetylcholine (ACH), histamine and bradykinin act as endogen mediators by activating membrane receptors leading to opening of ion channels. In the following intracellular calcium levels rise leading to activation of eNOS. Also changes in blood pressure, external mechanic influences and endothelial shear stress leads to production of NO. (2)

NO produced in endothelial cells can bind to, and by this activate, the enzyme guanylate cyclase (GC) in vascular smooth muscle cells. GC produces cyclical guanosine monophosphate (cGMP) from guanosine triphosphate (GTP). cGMP

mediates relaxation of smooth muscle cells in the tunica media, which results in dilated vessels (vasodilation). Degradation of NO happens within seconds, as it is a free radical and therefore reacts with different molecules. This explains its short distance of effect and the necessity of constantly new synthesized NO. Another endothelium derived molecule leading to vasodilation is prostacyclin (prostaglandin I_2, PGI_2). PGI_2 activates adenyl cyclase and by the increase of cAMP, a second messenger molecule, and by the activation of protein kinase A, vascular smooth muscle cells relax. (2)

Although not in the extend as in vasodilation, the endothelium secretes peptides causing vasoconstriction. These are not that relevant in short acting vasoconstriction but play an important role in pathophysiologies of e.g., in pulmonary hypertension. The peptide endothelin 1 (ET-1) is secreted by endothelial cells and leads to vasoconstriction by increasing the calcium level in vascular smooth muscle cells.(10)
As a consequence of endothelial shear stress, endothelial NADPH-oxidases generate superoxide anions, leading to an increased tone and proliferation in vascular smooth muscle cells (2).

Role in hemostasis

The most obvious role the endothelium plays in hemostasis is the one as a physical barrier (10). As an intact endothelium covers the subendothelial layers, neither subendothelial cells presenting tissue factor (TF) nor collagen fibers are exposed, not acting as a starting point for primary or secondary hemostasis (2).
Collagen fibers, exposed due to injured endothelium, act as a receptor for von Willebrand Factor (vWF). Binding of vWF to collagen fibers initiates primary hemostasis (11). While most coagulation factors (secondary hemostasis) are produced within the liver, vWF is synthesized in megakaryocytes and in endothelial cells (2). In case of injuries to the vessel, vasoconstriction as part of the primary hemostasis is initiated due to catecholamines (2), thromboxane (6) and other factors (thromboxane e.g.) secreted by activated thrombocytes in order to slow down blood stream velocity and following prevent greater blood loss (2). Additional vasoconstriction can caused by the peptide endothelin of which three forms exist within the human body. Only one of them, endothelin 1 (ET-1) is

synthesized by the endothelium (10). With the release of ET-1, smooth muscle cells within the vessel wall relax, resulting in vasodilation. However, ET-1 is thought not to have much credit in physiological regulation of the blood flow (6).
Further proteins or molecules synthesized by endothelial cells playing a role in hemostasis are PGI_2, plasminogen activator inhibitor 1(PAI-1), NO, and heparan sulfate. While both PGI_2 and NO promote vasodilation (6), they also have an inhibitory effect on platelet aggregation and platelet adherence to the vessel wall. This, however, requires an intact endothelial layer (10). Heparan sulfate on the outer endothelial cell membrane acts as an inhibitor of thrombin. (10) Therefore, an intact endothelium is essential in maintaining physiological hemostasis.
(For further description of hemostasis see 1.3)

Angiogenesis and wound healing

Vascular endothelial growth factor (VEGF) refers to a group of proteins promoting, among others, endothelial proliferation and vasodilation. In this context, especially the subtype VEGF-A is important. In metabolic high active tissues, such as growing or damaged tissue and also malignant tissues, VEGF is secreted to facilitate a better blood supply by vasodilation and angiogenesis (3). Endothelial cells have receptors VEGF binds to, signaling the start of further growth factor synthesis and initiating of NO.
Endothelial cells also synthesize and store P-selectin. After stimulation/activation through inflammatory cytokines, thrombin or histamine, endothelial cells present P-selectin on their membrane. P-selectin recruits leucocytes to the site of vascular injury or inflammation (2) (6). Interleukin-1 (IL-1), synthesized by endothelial cells, also leads to binding of leucocytes to receptors in the endothelial membrane (e.g. e-selectin) and enabling them to pass through the basal membrane into damaged tissue (12).

Endothelial dysfunction

Endothelial dysfunction refers to the shortage in NO synthesized and secreted by the endothelium. In consequence, the endothelium is not able to fulfill its regular NO-mediated functions (10) (2). Its vasodilative function is impaired due to a misbalance between vasodilative (NO) and vasoconstrictive substances. Further, the shortage in NO leads to an activation of the proinflammatory transcription factor NF-kB, leading to an increased expression of P-selectin and Interleukin-1

(2). As mentioned earlier, p-selectin and IL-1 promote adhesion of leucocytes and thus inflammation, arteriosclerosis, and thrombosis (2) (12). This is in amplification of the sequelae of NO shortage itself, as NO promotes the inhibition of monocyte- and thrombocyte adhesion to the endothelium. Increased levels of oxygen radicals within the vessel wall are thought to be the mean reason for the shortage in NO (2). Measuring endothelial function enables clinicians to identify preclinic stages of arteriosclerosis and therefore risks for related diseases like myocardial infarction, stroke or peripheral arterial occlusion. Common methods of assessing endothelial dysfunction is measuring the flow mediated dilation of the arteria brachialis or the reactive hyperemia (2).

1.2 Compromised Vascular Health: Vascular Diseases

To ensure vascular health, a healthy vascular system, fulfilling the required tasks, is crucial. In vascular disease, vascular function is compromised, effecting not only the vessel itself but potentially all other organs as well. Vascular disease includes many conditions; in the following conditions are described that are risk factors for vessel occlusion.

1.2.1 Inflammatory Diseases

Vasculitides are inflammatory diseases of the vessels that can be categorized by the vessels they affect and by their etiology (4). Vasculitides can be divided into primary and secondary ones (13). First emerge within the vessel wall itself, the second are a consequence to an underlying systemic or other local disease (4). All have an inflammation of the vessel wall, and in the following a potentially ischemia, and necrotic damage to the organ they provide with blood in common. Most primary vasculitides are due to an autoimmune pathology based on genetic factors, combined with environmental influences, while the specific etiology remains idiopathic (4). Depending on the size of affected bloodvessels, primary vasculitides are grouped into those affecting large, medium or small vessels (14). Large vessel vasculitides (mainly affecting the aorta and its big branches) are Giant cell arteritis and Takayasu arteritis. Medium vessel vasculitides affecting big and medium arteries are Kawasaki disease and Polyarteritis nodosa (PAN). In small vessel vasculitis one differs between those associated with anti-neutrophil cytoplasmic antibodies (ANCA) and those not associated with ANCA. Examples of

ANCA-associated small vessel vasculitides are Granulomatosis with polyangiitis, eosinophilic Granulomatosis with polyangiitis (Churg-Strauss syndrome) and Microscopic polyangiitis. Henoch-Schoenlein purpura (immunoglobulin A vasculitis), Cryoglobulinemic vasculitis, Cutaneous small vessel vasculitis and Behcet disease count towards the non-ANCA-associated vasculitides of small vessels (14). Some of the primary vasculitides are also associated with viral infections, like the giant cell arteritis (14). PAN is associated with hepatitis B (4) and cryoglobilenemia is associated with hepatitis C infections (14). Secondary vasculitides can be due to viral infections (like cytomegaly virus, parvovirus B19, human immunodeficiency virus or hepatitis-B- or -C-virus), bacterial infections (mostly pyogenic bacteria), fungal infections, parasites, chronic inflammatory diseases (systemic lupus erythematodes, sjörgen syndrome, primary biliary cirrhosis, inflammatory bowel diseases), tumors (acute myeloid leukemia, Hodgkin lymphoma, Non-Hodgkin lymphoma or carcinoma of liver, colon, kidneys), medications (penicillin, non-steroidal anti-inflammatory drugs (NSAID), carbimazol or thiouracil) or foreign proteins (like hyposensibilization antigens) (4) (13). Vasculitides can lead to an occlusion of the vessel and therefore in arteries a disruption in the perfusion of the organs downstream of it. In consequence, organ failure arise due to ischemia and necrosis (14). In venous thrombosis of superficial veins (for which varicosis is a risk factor), inflammation often occurs simultaneously, referring to thrombophlebitis. It is to note, that the inflammation can be both the underlying reason for the development of the thrombus or the consequence of it (13).

1.2.2 Arteriosclerosis

The term arteriosclerosis is used for degenerative processes of the arterial wall (4). There are mainly three types of arteriosclerosis: atherosclerosis, Mönckeberg media sclerosis and arteriolosclerosis (13). The first will be described further in the following. The second refers to calcification of the tunica media as a result of lipid depositions and occurs mainly in patients with diabetes mellitus type II or patients undergoing dialysis. The third describes lipid depositions in the tunica intima and media of small vessels, especially in kidneys, spleen and pancreas. Risk factors for developing arteriosclerosis are mainly life style dependent: arterial hypertension, hypercholesterinemia, cigarette smoke and diabetes mellitus (4).

Atherosclerosis

While there are many hypotheses regarding the development of atherosclerosis the exact pathogenesis remains uncertain (12). The conventional model describes it as a process starting in the inner lining of a vessel, the tunica intima or the endothelium, proceeding towards its deeper layers. A damaged endothelium marks the starting point for the development of atherosclerosis. This damage can be due to chronic endothelial stress caused by hemodynamic strain (arterial hypertension or at local predilection sites) or by biochemical-toxic strain (dyslipidemia, homocystemia, exogen toxins like nicotine, chronic inflammatory diseases or infections) (13) (12). A damaged endothelium means there is endothelial dysfunction. This term is used when it comes to a deficiency in NO, caused by an insufficient eNOS or by oxidative stress (2). In consequence of the endothelial dysfunction, monocytes, lymphocytes and platelets inhere on the damaged endothelium. Monocytes and lymphocytes will pass the damaged endothelial barrier while platelets release inflammatory mediators like cytokines, all promoting inflammation of the vessel wall, and platelet-derived growth factor (PDGF) (12). Smooth muscle cells (SMC) of the tunica media are stimulated by PDGF to migrate into the tunica intima and proliferate there, resulting in thickening of the tunica intima. Fibroblasts are also stimulated by PDGF to differentiate to myofibroblasts. With progression of the inflammatory process also the tunica media will be affected. The damaged endothelial barrier also enables LDL entering the vessel wall where it is oxidized by free radicals. Oxidized LDL induces release of cytokines which again enable adhesion of monocytes. Monocytes entering the tunica intima differentiate into macrophages which produce growth factors and cytokines and turn into foam cells by taking up LDL and oxidized LDL (12) (13). These foam cells add up and form the so called “fatty streak”, an early atherosclerotic lesion. SMC in the tunica intima as well as the macrophages produce extracellular matrix; these three compounds as well as lymphocytes develop a fibrous cap covering the layer of foam cells, cholesterol and cellular debris. This describes a fibrous plaque, an atheroma, with a fibrous cap and a necrotic center underneath (13).

Growth of the atheroma leads to reduction in vessel width, which can build up to a significant stenosis (12). As inflammatory cells within the atheroma produce enzymes breaking down the extracellular matrix, the fibrous cap becomes

weakened. This makes it easier to rupture due to already minor stress. In plaque rupturing the necrotic center will be exposed resulting in formation of a thrombus with following occlusion of the arteria or dispersion of thrombogenic material with the blood flow (13). Arising common complications from an occluded vessel due to atherosclerosis or following thromboemboli are coronary heart disease and acute myocardial infarction, stroke, and peripheral arterial disease (4) (12) (13). Figure 2(A) shows a normal artery in which normal blood flow is possible. Figure 2(B) shows an artery with an atherosclerotic plaque, narrowing the vessel width and hindering blood flow.

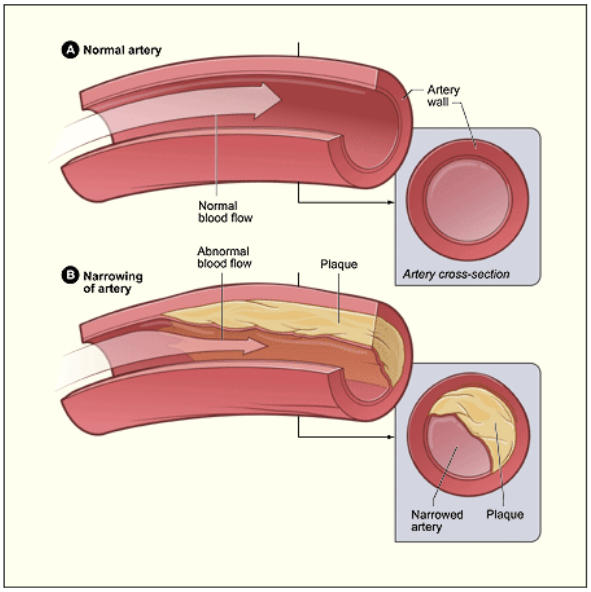

Figure 2: Blood flow within an artery: A) Normal artery with normal blood flow. B) Artery with atherosclerotic plaque (reproduced from (15))

While the mechanisms described above are seen as the conventional pathogenesis of atherosclerosis, there are also other hypotheses to its pathogenesis. In contrast to the development starting at the inner vessel wall, another less common hypothesis, postulated by Prof. A. Haverich (16), sees the beginning of atherosclerotic lesions at the outer vessel wall. In the tunica adventitia of large arteries smaller vessels can be found, so called “vasa vasorum”, which supply oxygen and nutrients to the vessel wall. If it comes to occlusion of the vasa vasorum, e.g., due to inflammation or microthrombi, ischemia of the larger artery will follow. It is then hypothesized that in part of the reparation process by the immune system, cellular debris would be generated accumulating in the vessel wall and forming a plaque, occluding the vessel (16).

1.2.3 Thrombotic/Thromboembolic Events

A thrombotic event or a thromboembolic event is referred to a thrombus or a thromboembolus blocking a blood vessel, interrupting a continuous blood flow, with resulting clinical consequences. (4,13,14,17)

Pathophysiology/ Etiology

A thrombus is a blood clot resulting from hemostasis within a blood vessel due to pathological changes summarized as the Virchow´s triad: vessel wall changes, hemodynamic changes or composition of the blood (13). The first includes structural and functional changes to the endothelium and the basal membrane of different etiologies: (ruptured) atherosclerotic plaques, mechanical stress (hypertonus), exogenic factors (chemotherapeutics, tabaco smoking, radiation or bacterial toxins), endogenous factors (hypoxia, hypercholesterinemia), inflammatory or immunological reactions. The second refers either to a slowed blood flow e.g. in pathological dilated vessels (aneurysm, varicose veins or dilated left atrium) or due to right heart insufficiency or to turbulences (13). The third includes changes in the blood cell count and blood composition of varies underlying pathophysiologies, all resulting in a hypercoagulable state. High cell count results in a greater viscosity in therefore in a greater risk for stasis, especially in small vessels. E.g., deficiency in anticoagulant factors, hereditary or acquired, like antithrombin deficiency, protein S or protein C deficiency, result in thrombophilia. Acquired Protein C and S deficiency can be a consequence due to liver cirrhosis. Hereditary causes of thrombophilia can be next to deficiency in antithrombin, in protein S or C, an APC resistance/ Factor V-Leiden mutation or a mutation in the prothrombin (factor II) gene (14).

Referring to the pathogenesis and morphology one differentiates a precipitation thrombus, a coagulation thrombus, a mixed thrombus, a hyaline thrombus, a tumor thrombus, and a postmortem clot. (13)

Arterial thrombosis is mainly caused due to rupture of an atherosclerotic plaque or due to inflammatory vascular processes. Also, certain rare diseases, like the antiphospholipid syndrome, or surgical complications can be responsible for arterial thrombosis. Arterial thromboemboli can be caused by cardiac arrhythmia, a travelling atherosclerotic thrombus, or in rare cases a venous thrombus entering the arterial system. (4) Venous thrombosis occur as a consequence of a slowed

blood flow (right heart insufficiency) and are most often located in deep veins (legs, pelvis). Inflammation of the venous vessel wall and varicosis are risk factors to the development of thrombosis with superficial veins (13).

Risk Factors

Conditions which display a predisposition especially for thrombosis of deep veins are a former thrombosis, immobility, thrombophilia (hereditary or acquired, as mentioned above), consummation of nicotine, obesity, hypertonia, high levels of cholesterol, systemic inflammation, malignoma, certain medications like estrogen or ovulatory inhibitors, heart insufficiency or respiratory insufficiency, pregnancy and postpartum period, chronic venous insufficiency, varicosis, or age above 60 years. (14) (18). Cardiac arrhythmia, artificial valves and valvular heart diseases, arterial aneurysms, polycythemia vera, or antiphospholipid syndrome are risk factors especially for arterial thrombosis and thromboemboli. (4)

Consequences

Depending on the location of a thrombus and its seize, it can block and occlude vessels. In venous occlusion, blood in the specific vein is inhibited from flowing back to the heart and builds up in the periphery (usually seen in swollen, blue extremities). A possible long-term consequence is the post-thrombotic syndrome, in which vessel damage, reduced blood flow and vascular inflammation persist. (4) (17). In arterial occlusion, the blood supply to the downstream organs is inhibited, resulting in ischemia. A thrombus travelling the blood flow from its location of origin to another location is called a thromboembolus, potentially causing a thromboembolic event. E.g., deep vein thrombi can embolize via the V. cava inferior into the pulmonary arteries causing a pulmonary embolism (PE). Thrombi within the coronary arteries, mainly in consequence due to atherosclerosis, can cause myocardial ischemia (MI). Thromboemboli within cerebral vessels (often a thrombus formed within the heart due to arrhythmia) can cause cerebral ischemia, an ischemic stroke. Arterial embolism can cause peripheral arterial occlusions resulting in clinical symptoms in the affected extremity such as heavy pain, paleness, pulselessness, pallor, paresthesia, paralysis and poikilothermia (4). The term venous thromboembolism (VTE) is usually used for both deep vein thrombosis (DVT) and pulmonary embolism (PE) (19).

1.3 Hemostasis

Processes within the body leading to cease a bleeding are referred to as hemostasis. One differentiates a primary hemostasis and a secondary hemostasis. While the differentiation and separation in primary and secondary hemostasis is claimed to be outdated it is still used in a didactive manner (4). Figure 3 shows on overview of the different parts of the hemostatic system.

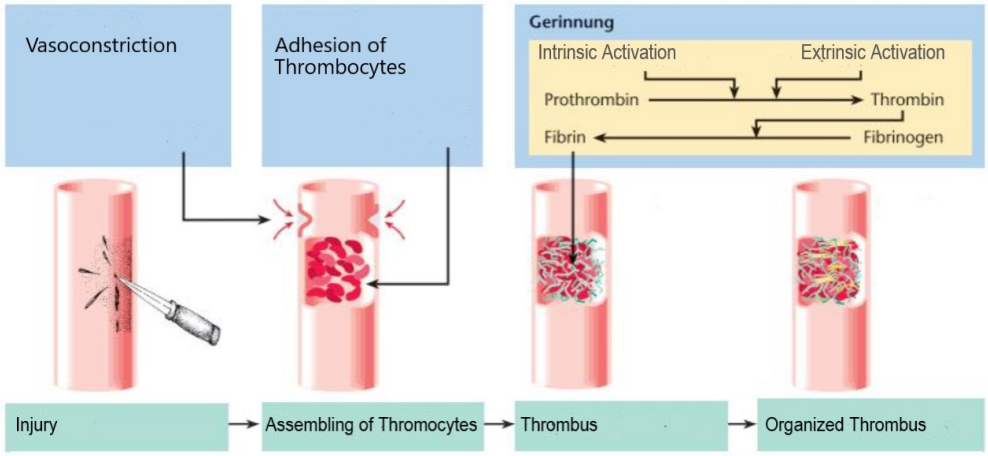

Figure 3: Primary and secondary hemostasis (modified after (4))

1.3.1 Primary or Cellular Hemostasis

Primary hemostasis refers to both vascular hemostasis, in which injuries to the endothelium lead to vasoconstriction, and to von Willebrand Factor (vWF) activation, with following platelet activation, resulting in formation of a so-called white thrombus. In the second, thrombocytes adhere via their glycoprotein (GP) Ib to vWF which is bound to the exposed vessel´s collagen. Via the GP IIa and GP Ia, thrombocytes can also directly bind to collagen (11). Following the adhesion, the thrombocytes get activated. This involves the change of the thrombocyte´s shape, the release of mediators from granules, leading to activation of more thrombocytes, and thrombocyte aggregation. The change of shape enables the aggregated thrombocytes to interlock with each other and leads to presentation of GP IIb/IIIa on the thrombocyte´s membrane (2). Fibrinogen binds to GP IIb/IIIa of activated thrombocytes and therefore connects them with each other (11) (Figure 4). Released mediators from thrombocytes´ granules include adenosine diphosphate (ADP), serotonin, coagulation factors (fibrinogen or factor I, factor V, factor VIII), vWF, fibronectin, thrombospondin and growth factors (transforming growth factor ß (TGF-ß), platelet-derived growth factor (PDGF), vascular

endothelial growth factor (VGEF) and basic fibroblast growth factor (bFGF)). Activated thrombocytes also synthesize thromboxane A_2 (TxA_2), which enhances further platelet activation and acts vasoconstrictive, and platelet activating factor (PAF), activating further thrombocytes and phagocytes (2). The connection of several thrombocytes leads to thrombocyte aggregation, or formation of the so called white thrombus.

With the endothelium being intact there is no activation of thrombocytes due to lack in exposed collagen and further due to thrombocytes inhibiting factors released by the endothelium: NO and prostacyclin (PGI_2) (2).

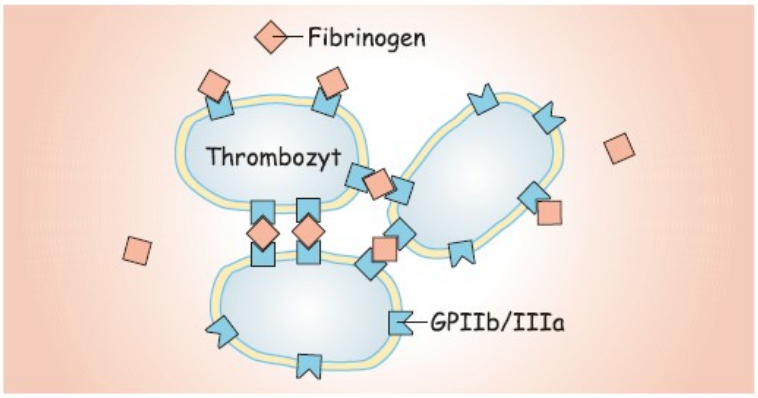

Figure 4: Aggregation of thrombocytes/platelets (reproduced from (10))

1.3.2 Secondary Hemostasis or Plasmatic Coagulation

Secondary hemostasis is also referred to as coagulation and describes the processes that lead up to stabilization of the instable white thrombus, forming a fibrin network with attached erythrocytes, forming the so-called red thrombus. The coagulation cascade can be activated endogenously by the intrinsic system, following endothelial injury, or exogenically by the extrinsic system, following injuries to the tissue. Both the intrinsic pathway and the extrinsic pathway end up in a common pathway by activation of factor X.

Extrinsic pathway

The extrinsic pathway is initiated by tissue factor (TF, factor III) synthesized by the subendothelial fibroblasts and vascular smooth muscle cells. Tissue factor is also synthesized in other cells like monocytes and tissues (lung, placenta) and is not only involved in coagulation but also in inflammation, apoptosis and cell migration (11). In injuries to the endothelium the subendothelial matrix gets exposed and TF gets in contact with factor VII, which is circulating in the blood, activating it to factor

VIIa. TF, VIIa, phospholipids and calcium build a complex, the so called extrinsic tenase (Figure 5). This complex activates factor IX and X. (10) (11)

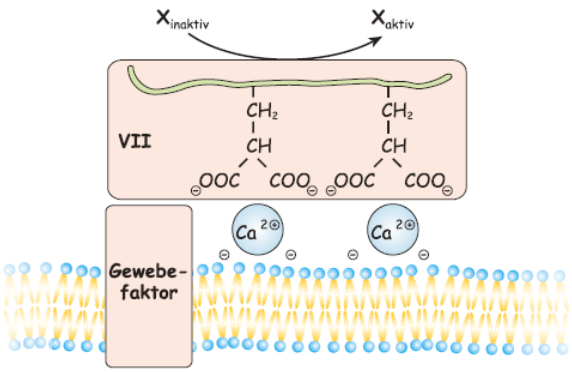

Figure 5: Extrinsic tenase (reproduced from (10))

Intrinsic pathway

The intrinsic pathway is not as important as the extrinsic pathway when it comes to initiating coagulation in vivo. It does play a role when blood comes in contact with foreign surfaces like it is the case e.g. on artificial valves or in laboratory settings (2). It is initiated by coagulation Factor XII getting in contact with negatively charged foreign surfaces and high-molecular-weight kinogen (HMWK) and kallikrein, leading to activated factor XII, factor XIIa (10). Factor XIIa then activates factor XI to factor XIa, followed by activation of factor IX to IXa by factor XIa (2). Factor IIa, thrombin, activates factor VIII to factor VIIIa. A complex consisting of factor VIIIa, factor IXa, Calcium (Ca2+) and phospholipids is built (2). This complex activates factor X to factor Xa (2).

Common pathway

Both the intrinsic and the extrinsic pathway lead, in addition with calcium, to activation of factor X. Together with factor Va, phospholipids and calcium, factor Xa builds a complex, the so called prothrombinase (seen in Figure 6) (10). This complex catalyzes the reaction from prothrombin (factor II) to thrombin (factor IIa) (10).

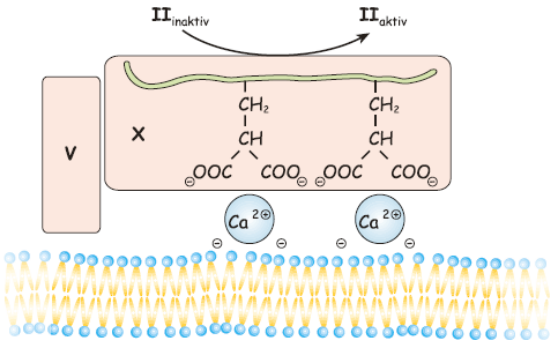

Figure 6: Prothrombinase (reproduced from (10))

Thrombin fulfills multiple tasks in hemostasis: in primary hemostasis it promotes synthesis of thromboxan and platelet aggregation and in secondary hemostasis it is responsible for turning fibrinogen into fibrin and for activating factor XIII (10). Factor XIIIa is needed for turning soluble fibrinpolymers into unsoluble ones (10). Furthermore, thrombin activates lymphocytes and acts a mitogen in endothelial cells and smooth muscle cells within the vessel wall (2).

Fibrinogen, a glycoprotein and also an acute phase protein, is synthesized in the liver. It builds up fibrinpolymers and connects activated thrombocytes with each other (10). Figure 7 shows a simplified scheme of the whole coagulation cascade.

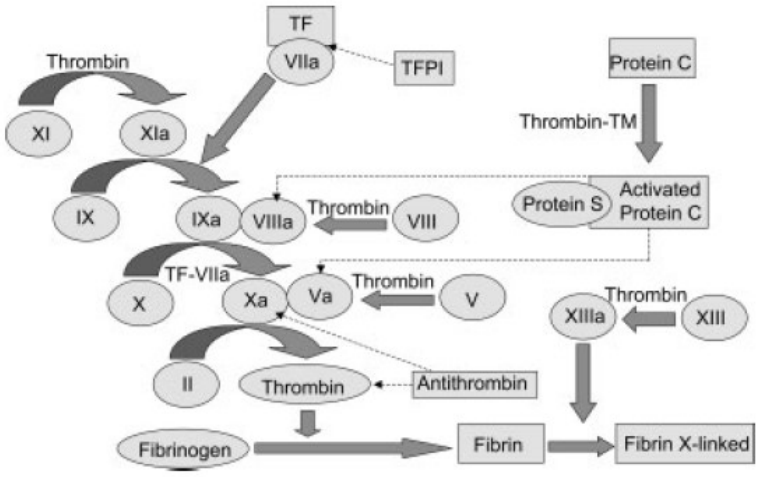

Figure 7: Simplified scheme of the coagulation cascade (reproduced from (20))

1.3.3 Cell-Based Model of Coagulation

The conventional model of the hemostatic process, with primary and secondary hemostasis and further with the intrinsic and extrinsic pathway, is nowadays seen as a more didactic than realistic model (4). A model integrating the concepts of primary and secondary hemostasis, while diverging from the strict separation of both in two different processes, is the cell-based concept (11). It proposes a better model of coagulation in vivo and sees the characteristics of different cell surfaces as regulative (21). There are three overlapping phases (not a cascade) of the cell-based concept (22).

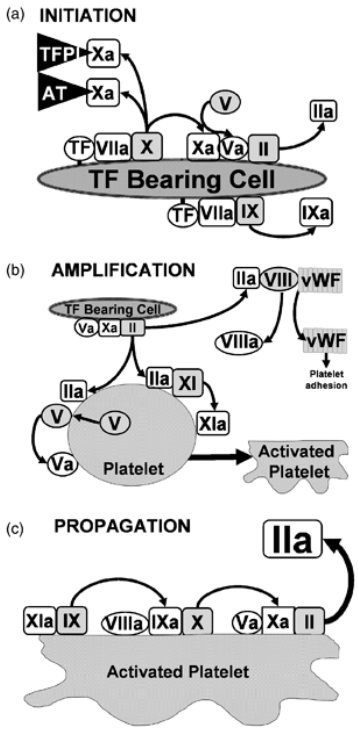

Figure 8: The cell-based model of fibrin formation (reproduced from (22))

Initiation phase

When tissue factor gets exposed after endothelial injury and therefore gets in contact with plasma, it binds to and activates factor VII. In the following, factor X, factor IX, and factor V get activated. Factor Xa and factor Va form a complex which initiates thrombin formation from prothrombin. (11) (Figure 8(a))

Amplification phase

The amount of generated thrombin is small, demanding activation of thrombocytes to ensure sufficient coagulation. Therefore, thrombin amplifies activation of

thrombocytes by activating factors V, VIII, and XI on the thrombocytes´ surface. (11) (Figure 8(b))

Propagation phase

The activated coagulation factors and complexes gather on surfaces of activated thrombocytes. After forming intrinsic tenase and prothrombinase, greater amounts of thrombin get generated (so called “thrombin burst”). (11) (Figure 8(c))

1.3.4 Anticoagulation Factors and Fibrinolysis

Antithrombin III

Antithrombin III (AT III) is a physiologically occurring anti-coagulation factor synthesized in the liver. It inhibits thrombin, factor Xa, IXa, XIa, and factor XIIa. (4)

Thrombin, thrombomodulin, protein C and S

On intact endothelium, thrombomodulin acts as a receptor for thrombin (factor II). By binding to TM, thrombin gets inactivated in its function to promote hemostasis but TM activates the protein C/protein S complex. The complex out of protein C along with its cofactor protein S (as seen in Figure 9) inactivates factor Va and factor VIIIa. (11)

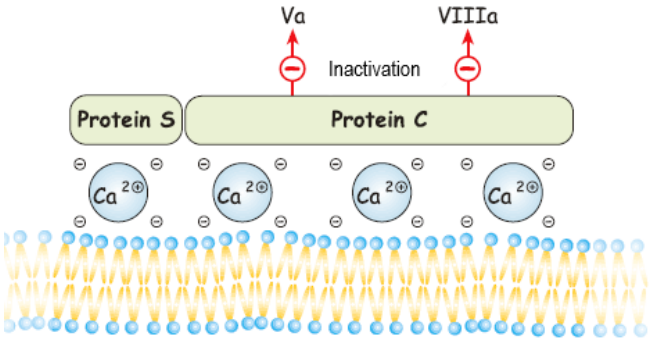

Figure 9: The protein C/protein S complex (modified after (10))

ADAMTS-13

The protein ‘A disintegrin and metalloprotease with thrombospondin-1-like domains” (ADAMTS-13) is a metalloprotease responsible for cleaving vWF and therefore acting as an anti-coagulation factor. (23)

Fibrinolysis

The process of resolving crosslinked fibrin is referred to as fibrinolysis. The enzyme plasmin binds to fibrin and is responsible for cleaving fibrin polymers into

smaller fragments. The proenzyme of plasmin is plasminogen, which gets activated by different physiological plasminogen activating factors, such as tissue-type plasminogen activator (tPA) (as seen in Figure 10), urokinase or oxygen deficiency (11). The glycoprotein tPA, which can also cleave vWF, is mainly synthesized by endothelial cells and is usually bound to its inhibitor PAI-1 (10).
Also therapeutic agents like streptokinase, urokinase or recombinant tPA (rtPA) result in fibrinolysis (11). Inhibitors of fibrinolysis are plasminogen activator inhibitor-1 (PAI-1) and alpha-2-antiplasmin (11).

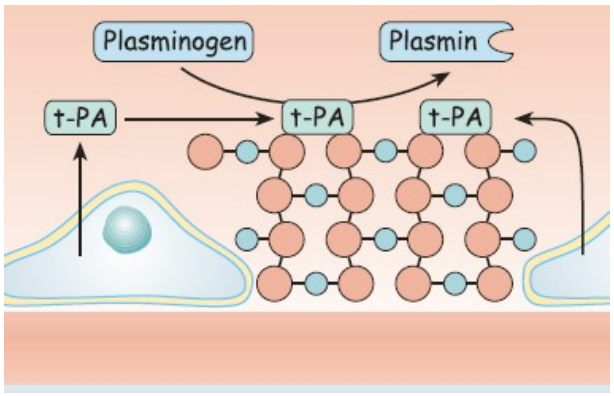

Figure 10: Activation of plasminogen to plasmin by t-PA (reproduced from (10))

1.3.5 Tests For Assessing Coagulation

1.3.5.1 Conventional Coagulation Tests

Conventional coagulation tests (CCT) are common in monitoring and evaluating a patient's hemostasis. They provide minimal labor requirements and are inexpensive but may have delayed turnaround times. (24)

Platelet Count (PC)

Number of platelets/thrombocytes per one microliter blood. Normal range 140-40 x 10^9/l. (4)

Prothrombin Time

Prothrombin time (PT) is the duration from the activation of the extrinsic system (in vitro through addition of thromboplastin) to the formation of fibrin polymers. It is used to evaluate the secondary hemostasis´ extrinsic and common pathway (factor VII, factor X, factor V, factor II/ prothrombin, fibrinogen). PT is prolongated in vitamin K deficiency and in deficiency of involved coagulation factors. PT is

clinically used to measure efficiency of vitamin K antagonist therapy (coumarins like phenprocoumon or warfarin). Normal range 70-120%. (4)

International Normalized Ratio

The international normalized ratio (INR) of the PT describes the factor the PT of a patient´s plasma is prolongated compared to the PT of normal average plasma (as defined by the WHO). Normal range 0.9-1.15. (4)

Thrombin Time

Thrombin time (TT) is the time from added thrombin (factor IIa) to formation of fibrin polymers. It therefore only evaluates the common pathway n secondary hemostasis and is used to test for fibrinogen deficiency or to evaluate a therapy with heparin. (4)

(Activated) Partial Thrombin Time

Activated partial thrombin time (aPTT) is the time from activation of the intrinsic pathway (in vitro through added phospholipids) to the formation of fibrin polymers. It is used to evaluate the intrinsic and common pathway in secondary hemostasis (factor XII, factor XI, factor IX, factor VIII, factor X, factor V, thrombin, fibrinogen).
aPTT is, e.g., prolongated in vitamin K deficiency or deficiency of involved coagulation factors. Clinically it is also used to monitor a therapy with heparin. (4)

Fibrinogen

Normal range 6-12mmol/l. Fibrinogen is decreased in hyperfibrinolysis or in disseminated intravascular coagulation (4).

1.3.5.2 Thrombin Generation Assay

In order to assess hemostasis more at its entirety, other tests than CCT have been developed (25). One of them is the thrombin generation (TG) assay (TGA), evaluating thrombin generation and decay (19). Parameters described in a thrombin generation assay (TGA) are (as seen in Figure 11): Endogenous thrombin potential (ETP), peak height (peak thrombin potential), lag-time, time to peak.

Endogenous thrombin potential

Tissue factor (TF) is given to a blood sample and the amount of thrombin getting produced in the consequence is measured. This amount of thrombin is called the 'endogenous thrombin potential' (ETP). It is thought, that the total amount of produced thrombin represents the balance of pro- and anti-coagulant factors (26). In hypercoagulability, ETP would be high. Vice versa, in hypocoagulability, ETP would be low (19).

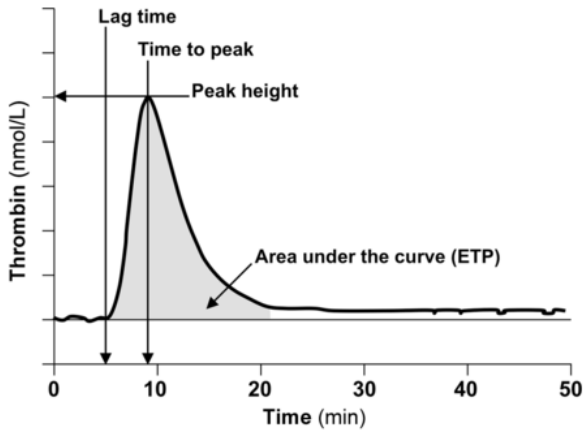

Figure 11: Thrombin generation assay (reproduced from (19))

1.3.5.3 Viscoelastic Tests

Viscoelastic tests (VET) refer to point-of-care tests (27) assessing clot dynamics and mechanics during all coagulation phases and thereby allowing a specific detection of coagulopathy (28) (29). Rational thromboelastometry (ROTEM®) and thromboelastography (TEG®) are the most common VET, with TEG® mainly used in the USA and ROTEM® mainly used in Europe (29). Both tests use whole blood

samples for measuring clotting initiating, clot kinetics, clot stability and clot break down. For the tests, a cuvette is filled with whole blood and a sensor pin is placed in the probe (28). In TEG® the cuvette is being alternate rotating by ± 4.75°, in ROTEM® the pin is alternate rotating. With progressing clot formation, the movement between cuvette and pin changes, giving the base for calculations of hemostasis. Figure 12 shows the setup of ROTEM®. As VET use whole blood samples, most components of coagulation are involved in the process: erythrocytes, leukocytes, platelets, and components of blood plasma. Therefore, VET represent more than just one pathway in coagulation but, respectively, intrinsic and extrinsic pathway as well as fibrinogen activity. This allows are more realistic result assessing in vivo functional coagulation compared to CCT. (29) (27) (24)

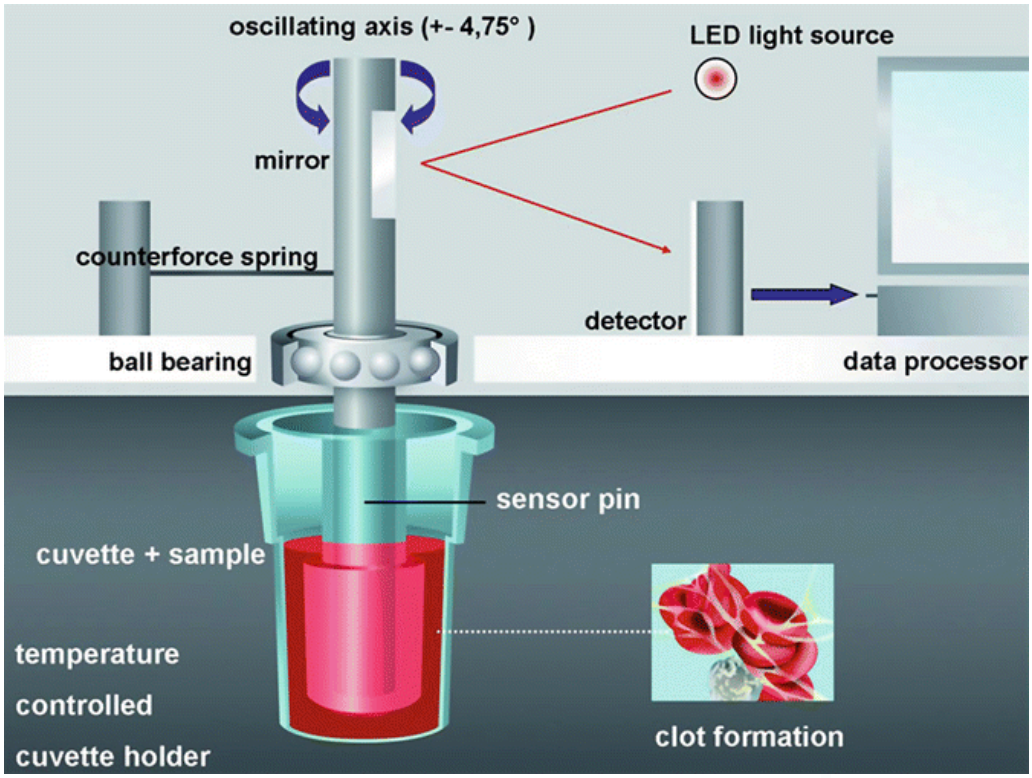

Figure 12: Setup of ROTEM® (reproduced from (30))

There are different assays available for ROTEM and TEG. E.g., EXTEM assay is testing extrinsic hemostatic function by activating hemostasis due to tissue factor, and INTEM assay is testing the intrinsic pathway, while FIBTEM analysis fibrinogen functionality or clot formation without the influence of platelets, which are inactivated by cytochalasin D. (31) (29)

Table 1 shows measurements by the two common viscoelastic tests ROTEM® and TEG® with interpretation regarding a hyper- or hypocoagulable state.

Table 1: Interpretation of viscoelastic tests (adapted from (32) and (28))

Result	ROTEM®	TEG®	Hypercoagulable	Hypocoagulable
Time until onset of fibrin formation	Clot time (CT)	Reaction time (R)	Short R or CT	Long R or CT
Rate of fibrin formation	Clot formation time (CFT) and α	K and α	Short K, high α, short CFT	Long K, low α, long CFT
Maximum strength of the fibrin clot	Maximum clot firmness (MCF)	Maximum amplitude (MA)	High MA or MCF	Low MA or MCF
Stability of fibrin clot	CLI-30 CLI-60	Ly30 Ly60		

Measurements obtained by TEG® are converted to a fibrin formation curve (as seen in Figure 13). The alpha angle marks the rate from clot formation initiation to maximal clot firmness (24).

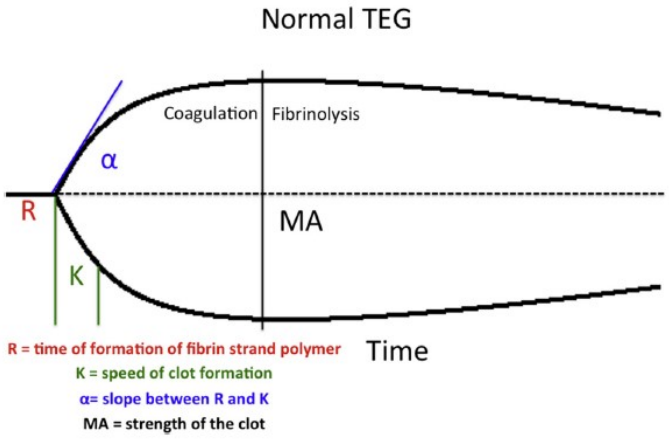

Figure 13: Example of a normal TEG (reproduced from (33))

Comparison of VET and CCT

While CCT have been standard tests to evaluate coagulation or efficiency of anti-coagulative therapies in everyday clinical life, VET were initially used in trauma to evaluate the need for transfusions (33). With VET being able to ass coagulation more globally than CCT, it became more used outside of trauma as well. Table 2 shows the direct comparison of CCT and VET.

Table 2: Comparison of VET and CCT (adapted from Cohen et al. (24))

	VET	CCT
Turnaround	Rapid (usually <30min)	Slower (usually >1h)
Costs	More expensive reagent and labor costs	Less expensive reagent and labor costs
Substrate	Whole blood	Blood plasma
Centrifugation	Not needed	Needed
Usage/Indication	Associated with less product usage and better outcome in acutely bleeding patients	Best suited as anticoagulant screening test in stable, nonbleeding patients
Detection of hyperfibrinolysis	Possible	Not possible
Needed devices	One device for evaluating clotting factors, PLTs, and fibrinogen	Multiple devices for evaluating clotting factors, PLTs, and fibrinogen
Relation to hemostasis	More closely resembles in vivo hemostasis	Highly artificial measurement of hemostasis
Measurement	Total clot strength	Clot initiation, no clot strength detectable

1.4 The Liver

With 2-5% of the bodyweight, the liver marks the second largest organ as well as the biggest gland within the human body (2). It is located in the right upper quadrant of the abdomen and can be divided into four lobes, anatomically seen, and in eight segments, functionally seen (1). Figure 14 shows the segments I, II, III, IVa, IVb, V, VI, VII and VII of the liver, as well as the ligamentum falciforme, which is, anatomically seen, dividing the left from the right lobe (34).

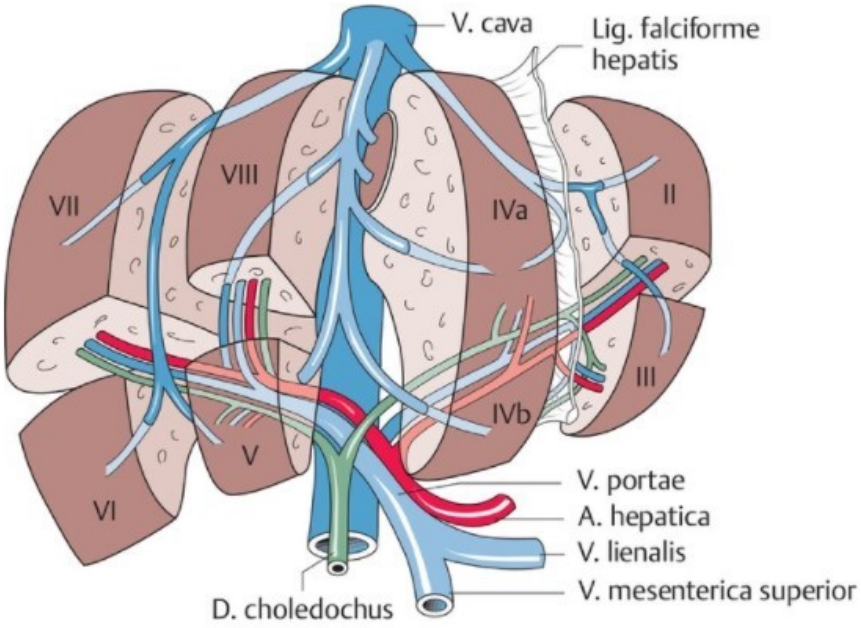

Figure 14: Anatomy of the liver (reproduced from (34))

The main functions of the liver are (6) (10) (2):

- Energy metabolism

  Glycolysis, ß-oxidation, proteolysis and amino acid degradation, breakdown of fructose and serotonin
- Synthesis

  Gluconeogenesis, production of ketone bodies, plasma proteins (e.g., albumin, globulins, acute phase proteins, clotting factors), cholesterol biosynthesis, bile and bile acids, fatty acids, Vitamin D
- Regulatory function

  E.g., keeping glucose homeostasis (glycogenolysis/glycogen synthesis, gluconeogenesis/glycolysis, insulin degradation)
- Storage

  Glycogen, lipoproteins, vitamins (A, B_{12}, E, D), folate, iron, copper

- Detoxification/biotransformation and excretion

  The urea cycle, in which ammonia is eliminated from the human body, is exclusive to the liver. The enzyme system cytochrome-P450 (CYP450) can be found in all organs but it is most frequently located within the liver. CYP450 is involved in many processes as it converts lipophilic substances into water-soluble ones.

The majority of liver cells, approximately 80%, are hepatocytes (2). They make up a one layered polarized epithelium without a basal membrane, are connected via tight junctions and are place to many metabolic processes (3). Nutrient rich blood coming from the gastrointestinal tract and the spleen is brought to the liver via the portal vein and will reach the hepatocytes via large capillaries, the sinusoids, which also contain blood from the A. hepatica (4) (10). The sinusoids endothelium is highly fenestrated and allows nutrients and other molecules to pass from the blood into the plasma filled perisinusoidal space (of Disse), which faces the basolateral site of hepatocytes (3) (2). In the other direction molecules secreted by the hepatocytes reach the blood stream of the sinusoids via the perisinusoidal space. Within the sinusoids liver specific macrophages, Kupffer cells, are responsible of clearing up bacteria or damaged blood cells (3). In the perisinusoidal space hepatic stellate cells (HSC, Ito cells) can be found. HSC contain vitamin A (3), are involved in development of fibrosis as they produce extracellular matrix and collagen, act in immune regulation and are able to transdifferentiate into myofibroblasts (2). The sinusoids enter into the central veins, which continue to enter into the V. cava. The apical site of the hepatocytes faces the bile canaliculi in which the produced bile will be secreted. Bile canaliculi form intrahepatic bile ducts, which then form the common bile duct. (3)(2)

1.4.1 Metabolism within the Liver

The liver is responsible for many metabolic processes and is therefore very active. It is responsible for 20% of consumed oxygen in rest and receives approximately 25% of the cardiac output (6) (4). Nutrients absorbed in the small intestine are transported via the portal vein to the liver.

Glucose

Glucose, as the most important energy supplier in the human body, is handled based on the metabolic phase the body is in. In the digestive phase the insulin level is high, resulting in glucose stored as glycogen or metabolized to pyruvate or fat. Is the insulin level low, as in the interdigestive phase, glucose is provided by the liver by running gluconeogenesis and glycogenolysis. (2)

Proteins and amino acids

Proteins synthesized by the liver make up 15-50g daily (2) and include plasma proteins (such as albumin and globulins), clotting factors (glycoproteins), carrier-proteins (e.g. transferrin, haptoglobin, ceruloplasmin), prohormones (angiotensinogen) and apolipoproteins. Amino acids are deaminized to keto acids and ammonium (NH_4^+) within the liver. Keto acids are then used in the citrate cycle to synthesize adenosine triphosphate (ATP). Also glutathione (GSH, a tripeptide), which reduces free oxygen radicals, is synthesized mainly in the liver. (2) (10)

Urea cycle

The urea cycle, a biochemical cascade in which urea is built from ammonia and bicarbonate, is specific to hepatocytes. Ammonia in the breakdown of glutamate, the product of amino acid degradation. Urea is, in contrast to ammonia, not toxic and can safely be transported via the bloodstream to the kidneys and be secreted with the urine. There are in total five reactions of which three are located within the hepatocyte´s cytosol and two within the mitochondrial matrix.

The participating molecules arginine, arginosuccinate, citrulline and ornithine leave the urea cycle unchanged when looking at its overall balance. (10)

Lipoproteins and triacylglycerol

In enterocytes of the small intestine, triacylglycerol (TAG) and cholesterol ester get reassembled from resorbed dietary fatty acids (4). TAG serve as an energy reservoir and are esters of three fatty acids and one glycerol molecule. As TAG and cholesterol are not water-soluble, they are transported via lipoproteins: hydrophile lipids (phospholipids and cholesterol) and hydrophile proteins (apolipoproteins, Apo-) on the surface, hydrophobic lipids (TAG and cholesterol ester) on the inside. There are five major lipoproteins, classified by their

physicochemical characteristics, their size and concentration and composition of lipids and apolipoproteins (2):

- Chylomicrons
- Very low density lipoprotein (VLDL)
- Intermediate-density lipoprotein (IDL)
- Low-density lipoprotein (LDL)
- High-density lipoprotein (HDL)

Chylomicrons transport dietary lipids from the small intestine to the periphery.
In muscle and fat tissue the capillary lipoprotein lipase splits fatty acids off of TAG from the chylomicrons. The fatty acids are being taken up by muscle and fat cells (2). The remnant chylomicrons (including TAG, cholesterol and cholesterol ester) are being taken up by the hepatocytes by receptor mediated endocytosis (LDL- and LDL-related receptor) (10). TAG are split into free fatty acids and glycerol (10).

Alcohol oxidation

After consumption, ethanol gets oxidized to acetaldehyde by the enzyme alcohol dehydrogenase (ADH) (35). Hepatic ADH is the most important when it comes to alcohol degradation, but it is also active in the intestines, kidneys and lungs. If the blood alcohol level is too high, another enzymatic system becomes active: the microsomal ethanol oxidizing system (MEOS) which depends on cytochrome P450 (36). Acetaldehyde is oxidized to acetic acid by the enzyme aldehyde dehydrogenase (ALDH). Both ALDH and ADH use nicotinamide adenine dinucleotide (NAD^+) as a coenzyme (36). Acetic acid gets activated to acetyl-coenzyme A (acetyl CoA) by the enzyme thiokinase (acetyl-CoA-synthetase). Acetyl CoA is an important substrate in many metabolic processes. It is converted to carbon dioxide and among others to energy in form of GTP in the citric acid cycle. Also, it serves as a substrate in the biosynthesis of fat and cholesterol. The limiting factor of alcohol oxidation is the reoxidation of NADH to NAD^+ (36). With higher levels of alcohol the $NADH+H^+/NAD^+$ quotient gets increased leading to a number of consequences: triglyceride synthesis increases, pyruvate is reduced to lactate in a higher ratio, gluconeogenesis, glycolysis and tricarboxylic acid cycle are inhibited. These effects lead to a lower blood glucose level, followed by in an increase in lipolysis and therefore in synthesis of ketone bodies resulting in an acidotic state. (35) (36)

1.4.2 The Role of the Liver in Hemostasis

The liver is involved in synthesis of procoagulant and anticoagulant factors, as well as in fibrinolytic processes. Next to the production of proteins necessary for hemostasis, the liver is also crucial for the clearance of activated clotting factors and the by-products of fibrin degradation. (37)

Table 3 gives an overview of proteins involved in hemostasis synthesized by the liver.

Table 3: Hepatic synthesized proteins acting in hemostasis (adapted from (37))

Protein	Function	Vitamin K-dependent	Inhibitors synthesized by the liver
Coagulation factors			
Factor II = Prothrombin		x	Antithrombin, Protein C, Protein S, TFPI, Heparin cofactor II
Factor VII			
Factor IX			
Factor X			
Factor V	Cofactor in thrombin activation		
Factor VIII	Cofactor in Factor X activation		
Factor XI			
Factor XII			
Factor XIII	Fibrin clot stabilization		
Fibrinogen (factor I)	Activated by thrombin to form fibrin		
Prekallikrein			
High-molecular-weight kininogen (HMWK)			
Anticoagulants			
Protein C	Activated by the binding of thrombomodulin to thrombin (IIa) to inactivate factors Va and VIIIa	x	
Protein S	Co-factor of protein C	x	
Protein Z	Interacts with protein Z-dependent protease inhibitor by acting to downregulate Factor Xa	x	
Fibrinolytic system			
Antithrombin III	Inhibition of thrombin		
Alpha-2-antiplasmin	Inhibition of plasmin		
Plasminogen	Binds to fibrin and tissue plasminogen activator to form plasmin which cleaves fibrin and releases fibrin degradation products		Plasmin inhibitor
Platelet function			
Thrombopoietin	Regulation of platelet production		
ADAMTS-13 *Predominantly synthesized in hepatic stellate cells and in endothelial cells*(38)	Cleavage of vWF, to promote bleeding		

1.4.3 Liver Diseases

There are manifold diseases to the liver of different etiologies. As it would exceed this thesis, only most common hepatocellular diseases which potentially require hepatectomy at a certain stage of disease, will be described further.

1.4.3.1 Hepatic Steatosis

Hepatic steatosis, or commonly known as fatty liver disease, describes a status with an excessive fat deposition in the liver. The underlying pathogenesis can be classified as either alcohol-toxic or non-alcohol-toxic. Hepatic steatosis can progress into steatohepatitis and while these two states are generally reversible, it can progress further into fibrosis and cirrhosis, which are nonreversible stages in liver disease and display a high-risk factor for hepatocellular carcinoma. (13)
In hepatic steatosis hepatocytes present with a disturbed metabolism of fatty acids and triglycerides. While the synthesis, storage and uptake of free fatty acids from the blood is increased, the fatty acid oxidation by the mitochondria and the secretion (in form of lipoproteins) in the blood is decreased. This leads to accumulation of fatty acids, stored in vacuoles, in the hepatocytes´ cytosol. (13)

1.4.3.2 Nonalcoholic Fatty Liver Disease

The term “nonalcoholic fatty liver disease” (NAFLD) includes nonalcoholic isolated hepatic steatosis (NAFL), nonalcoholic steatohepatitis (NASH), nonalcoholic hepatic fibrosis and cirrhosis. While the exact mechanisms in the pathogenesis of NAFLD are not completely understood (39) , there are certain health conditions it can be associated with. Many of these are metabolic disorders (like type 2 diabetes mellitus, glycogen storage diseases, lipodystrophy, abetalipoproteinemia or acute fatty liver of pregnancy), nutritional factors (malnutrition, obesity, refeeding syndrome, or total parenteral nutrition), hepatotoxic medication (e.g. methotrexate, glucocorticoids, amiodarone, diltiazem or antiretroviral therapy) or other diseases (like celiac disease, inflammatory bowel disease, hepatitis C or HIV) (40) (14). Especially insulin resistance, occurring in type 2 diabetes mellitus, is believed to play an important role in the pathogenesis of NAFLD (40). NAFLD is referred to as the hepatic manifestation of the metabolic syndrome (41).

When it comes to degradation of the stored fatty acids within the hepatocytes lipid membranes are destroyed, ultimately leading to degradation of the hepatocyte itself (hepatocellular ballooning degradation), and lobular inflammation: Steatosis (NAFL) has proceeded to steatohepatitis (NASH). While these two states are potentially reversible, NASH can proceed to chronic NASH in which it comes to accumulation of fibrotic tissue within the liver causing liver fibrosis, are generally not reversible state of liver disease. With proceeding liver fibrosis, more liver cells become necrotic and regular liver lobe structure is destroyed, marking the state of liver cirrhosis. (14)

1.4.3.3 Alcoholic Fatty Liver Disease

In Middle Europe, North and Middle America the majority of liver diseases is caused by chronic alcohol consumption (4). While there are individual levels of toxic alcohol amounts, low risk alcohol consumption is commonly defined as approximately less than 24g per day in men and 12g per day in women (14) and the critical/toxic amount of daily alcohol intake is commonly defined as approximately 60-80g in men and 20-40g in women (13). In chronic consumption of critical/toxic amounts of alcohol, approximately 30% of people develop an alcohol steatohepatitis (14) and approximately 25% develop liver cirrhosis (13). The oxidation of alcohol is described in 1.3.1. The metabolite acetaldehyde generated in alcohol oxidation is hepatotoxic as it binds to phospholipids, hormones, amino acids, cell membranes and other components like microtubules, it increases collagen synthesis and lipid peroxidation, activates complement factors as well as it interferes with mitochondrial transport of electrons (13). Acetate, the metabolite of acetaldehyde, is converted into acetyl-CoA. In chronic consumption of alcohol, Acetyl-CoA contributes to an increased synthesis of fatty acids and TAG. Also, it is assumed that alcohol inhibits hepatic release of lipoproteins; these mechanisms lead to hepatic steatosis (10). Histologically steatosis hepatis is differentiated into three degrees based on lipid depositions in hepatocytes; First degree or mild steatosis with less than 1/3 of hepatocytes affected, second degree or moderate steatosis with <2/3 of hepatocytes affected and third degree or severe steatosis with >2/3 of hepatocytes affected (14). Analogue to the pathophysiology in non-alcoholic fatty liver diseases, hepatic steatosis can progress with persistent damage to hepatocytes to alcoholic

steatohepatitis (ASH), hepatic fibrosis and finally cirrhosis (14). Also NADPH is postulated to play a crucial role in the pathomechanism of alcoholic fatty liver diseases as it leads to an increase in lactate and followed by inducing fibrosis.
However, the exact pathomechanism of alcohol mediated liver diseases is still disputed. (4)

1.4.3.4 Hepatitis

Hepatitis describes an inflammation of the liver. Hepatitides can be roughly be divided into viral and non-viral ones, with viruses being the most common underlying etiology. Bacteria, fungi or parasites are less common etiologies. Also (auto)immune reactions, toxins or metabolic disorders can lead to a hepatitis. The viral hepatitides can further grouped by the underlying viruses: hepatotropic viruses (hepatovirus A-E) and non-hepatotropic viruses (e.g. CMV or EBV). Hepatitides can either be acute or chronic with some of the acute forms progressing into chronic stages. Both acute and chronic stages can lead to liver cirrhosis. (4)

1.4.3.5 Liver Cirrhosis

Ranked by the WHO - Global Health Estimate (in 2016) (42) the 12th most common cause of death worldwide, liver cirrhosis marks an irreversible damage to the liver, following chronic liver disease with liver fibrosis. While the last is characterized by proliferation of fibrotic tissue (mostly collagen type 1) with obtained lobular structure, cirrhosis is characterized by liver cell necrosis and destruction of lobular architecture (4). Cirrhosis can be classified by morphology and etiology. Macroscopically a micronodular and a macronodular type are distinguished. In the microscopical type, regenerative nodes up to 3mm in diameter with no lobular structure or central vein can be seen. It usually follows chronic processes with slow progression like in alcoholic steatohepatitis, chronic hepatitis B or C. In the macroscopically type regenerative nodes with more than 3mm up to a few centimeters in diameters with portal fields and efferent veins are seen (13). There is also a type with components of both the microscopical type and the macroscopical type. In all types, nodes are surrounded by fibrotic septa. With histological identified progredient damages to the parenchyma, like necrosis

and inflammatory infiltrates, cirrhosis is classified active or progredient, whereas with no signs of active progression it is classified inactive or stationary (13). Liver cirrhosis can be the result of many different liver diseases. All have a distinct and long lasting damage to liver cells leading to necrosis and destruction of lobular architecture in common. The most common type of liver cirrhosis is due to chronic alcohol consumption (in industrial countries 50-60%), followed by metabolic syndrome or NASH (in industrial countries 10-20%), hepatitis (10-15%), biliary (5-10%), hemochromatosis (5%), idiopathic (<5%), metabolic diseases (<1%) and toxins (<1%) (13). Clinically liver cirrhosis marks an irreversible stage of disease or end-stage liver disease that, without liver transplantation being transformed, leads to death (43). The stages of liver cirrhosis are clinically classified by the Child Pugh Score (Table 4). One year survival rate in Child A is almost 100%, in Child B approximately 85% and in Child C approximately 35% (14).

Table 4: Child Pugh score for cirrhosis mortality (modified after (14))

	1 Point	2 Points	3 Points
Albumin (g/dl)	> 3,5	2,8 – 3,5	< 2,8
Bilirubin (mg/dl)	< 2,0	2,0 – 3,0	> 3,0
Bilirubin (µmol/l)	< 35	35 – 50	> 50
Bilirubin in PBC and PSC (mg/dl)	< 4	4 – 10	> 10
Bilirubin in PBC and PSC (µmol/l)	< 70	70 – 170	> 170
Quick (%) or INR	>70 / < 1,7	40 – 70 / 1,7-2,3	< 40 / > 2,3
Ascites (sonography)	Absent	Slight	Moderate
Encephalopathy	0	I – II	III - IV
Points added: Child A = 5 – 6 Child B = 7 – 9 Child C = 10 – 15			

1.4.3.6 Hepatocellular Adenoma

The hepatocellular adenoma is a benign tumor of the hepatic parenchyma. While the tumor itself is benign, it can lead to hemodynamic bleedings and compression of surrounding hepatic tissue. Proceeding into a hepatocellular carcinoma is rare but possible. In hepatocellular adenomas exceeding 5cm in diameter, a partial hepatectomy can be discussed. (13)

1.4.3.7 Hepatocellular Carcinoma

The hepatocellular carcinoma (HCC) is a malign primary tumor of the hepatic parenchyma (13). The etiology of HCC is diverse: Liver cirrhosis, as end-stage live disease of many etiologies, is seen as a precancerosis for HCC and is the

underlying etiology in over 90% of HCC (14). Thus, many liver diseases ending in cirrhosis (e.g., chronic alcoholic liver diseases, NAFLD, chronic hepatitis B and C) can proceed to HCC. Even without cirrhosis, hepatitis infections, mostly chronic type B or C, can lead to HCC (44). Especially hepatitis B seems to increase the risk of developing HCC (13). As in chronic hepatitis C, 30% of patients develop liver cirrhosis, of these 1-3% develop HCC (4). Also toxins (such as aflatoxins, arsenic, vinyl chloride) steroids, and (hereditary) metabolic disorders (NASH, haemochromatosis, α1-antitrypsine-deficiancy) can lead to HCC. Chronic liver diseases are responsible for approximately 90% of HCC (14). Macroscopically, there are three types of HCC differentiated: a massive, a multinodular and a diffuse type (13). In the first there is one big tumor seen, in the second several nodes can be detected and in the latter is characterized by a diffuse infiltration of the hepatic parenchyma (13). Typically, the HCC metastasizes easily, especially into lungs, bones, skin and lymph nodes (13). Therapy of HCC depends on the underlying disease and progression of the HCC, as categorized by the TNM-classification (14). In a curative setting a resection is possible under the following conditions: R0-resectable tumor, bilirubin in normal range, no metastasis, sufficient liver function (not worse than Child-Pugh A), and no portal hypertension. In liver cirrhosis with a single node less than 5cm in diameter or a maximum of 3 nodes with each less than 3cm in diameter, and no metastasis, a transplantation is possible. Also other curative procedures, such as radio-ablation are possible under certain conditions (14).

1.4.3.8 Liver Metastases

The most common malignant lesions within the liver are not primary ones but metastases (45). Liver Metastases most commonly originate from colorectal carcinoma (CRC), malignancy of the lungs, breasts, and upper gastrointestinal tract (13). In CRC, the liver is the most common location for metastases; every second patient shows hepatic metastases (14). Surgical resection is performed in primarily resectable liver metastases as well as in secondary resectable ones. There are also different therapy modalities available, such as adjuvant/neoadjuvant chemotherapy (45).

1.4.4 Liver Surgery

Liver surgery is commonly performed due to benign or malignant liver lesions, diagnostic purposes, organ transplantation, or due to traumatic injuries (45).

Hepatectomy

Liver resection, hepatectomy, refers to the surgical removal of parts of the liver parenchyma or even the whole liver itself. Partial hepatectomy is usually performed due to hepatic tumors with metastasis from colorectal cancer being the most common. Other common reasons for hepatectomy are HCC, intrahepatic cholangiocarcinoma, hemangioma of the liver, liver cysts (due to echinococcus e.g.), and focal nodular hyperplasia (FNH). Due to the great recovery capacity of the liver, one can resect a large percentage of the organ with still a preserved function and prospect of regaining a great amount of function back (45).
While there are different values of minimum needed remnant liver volume found in literature, it seems like 30% of liver function after hepatectomy is the most frequently referred one (46).

Liver transplantation

Reasons for liver transplantation cover a wide range of end-stage acute or chronic liver diseases including cholestatic diseases, liver tumors, primary and secondary metabolic diseases, vascular diseases and immense traumatic liver injuries (46). Liver grafts can be obtained both from living or deceased donors.

1.5 Hemostasis in Liver Disease

When reviewing the tasks of the liver, one can easily assume that changes in liver function will have an influence on hemostasis. Due to liver disease accompanying decrease in hepatic cell function it could be postulated that the rate of coagulation factor synthesis is decreased, resulting in a hypocoagulable state. On the other hand, it could be postulated that also the hepatic synthesized anticoagulant factors, fibrinolytic factors, as well as the clearance of activated factors are decreased, resulting in a hypercoagulable state. Taken these two arguments together, one could either assume that the overall result is a balanced state in hemostasis or that one or the other effect is dominating, resulting in disturbed hemostasis.

Historically, patients with liver diseases were seen as “auto-anticoagulated” with a tendency to bleedings (47). This was postulated due to clinically seen bleedings in patients with liver failure and due to prolonged PT and INR (48). However, in the early and mid-2000s more studies were conducted on hemostasis in patients with liver disease showing a fragile but rebalanced hemostatic system. Both procoagulant and anticoagulant factors as well as platelets were found to be decreased in liver disease (49) (50) (48). Even though these deficiencies result in an overall rebalanced hemostasis, the balance is not as stable as in healthy people. The balanced state might easily change towards hypo- or hypercoagulation with following bleeding or thrombosis/thromboembolism depending on individual risk factors (50). Figure 15 shows changes in hemostatic factors due to liver disease, promoting either bleeding or clotting: Synthesized products of the liver, such as coagulation factors II, V, VII, X and XI, fibrinogen, as well as he anticoagulants ADAMTS-13, protein C and S, and antithrombin are decreased with impaired liver cell function. A decreased platelet count and platelet activation is mostly due to hypersplenism (splenomegaly as a consequence of portal hypertension in liver cirrhosis) resulting in damaged thrombocytes (51). However, factor VIII, which is produced by the liver as well, is not seen to be decreased in liver disease. This can be explained by first extrahepatic sites of production, such as the endothelium, lungs and spleen, and second, and most importantly, due to its binding to vWF (52).

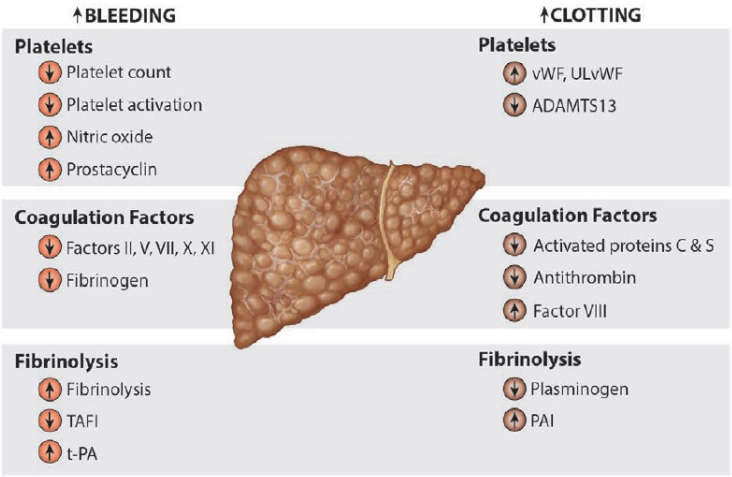

Figure 15: The coagulopathy of liver disease (reproduced from (52))

CCT, especially PT/INR and aPTT, were found to be no sufficient laboratory measurements to identify a state of hypo- or hypercoagulation in patients with liver disease (48). This is because PT/INR and aPTT only refer to procoagulant factors

but not on anticoagulant factors, which are as well decreased in liver disease. Even with prolongated PT/INR a patient can show a hypercoagulable state. It is therefore outdated to correct a prolongated PT/INR with fresh frozen plasma (FFP) in stable patients (48) (53) (19). Table 5 shows laboratory tests and their suitability to evaluate bleeding risk in liver disease.

Table 5: Clinical laboratory testing to evaluate bleeding risk (reproduced from (48))

Test	Current state of the art
Prothrombin time and INR	Unreliable as indicators of bleeding risk
Platelet count	Reliable, although not substantiated by data from clinical trials (no accurate threshold levels are available)
Skin bleeding time	Unreliable
Fibrinogen	Commonly used with target of >120 mg/dl or >150 mg/dl. Understandable rational (needed for fibrin formation) but lacking good clinical trials
Thromboelastometry/thromboelastography	Promising, but require further evaluation. Learning curve is evident for test interpretation
Thrombin-generation assays	Very useful research tool. Promising in clinical setting but require further evaluation
Fibrinolysis (tests for hyperfibrinolysis)	Promising, but standardized and reliable global assays are not widely available and require further evaluation. Responsiveness for hyperfibrinolysis detection may vary between different global assays

Additional to CCT, other tests were used to analyze hemostasis in liver disease further. E.g., Tripodi et al. (20) were among firsts to use thrombin generation assays (TGA) to assess hemostatic changes in cirrhotic patients. At first, they did not add thrombomodulin (TM) to the TGA and could show that in liver cirrhosis, thrombin generation was lower than in non-cirrhotic patients. Second, they performed TGA with added TM and thrombin generation was shown to be unchanged. TGA with added TM in non-cirrhotic probands was shown to be significantly reduced. In TGA without TM, thrombin generation is lower due to the decrease in coagulation factors, with added TM the process is not changed due to the decrease in protein C. In non-cirrhotic probands, a normal level of protein C, which gets activated by TM, results in a reduced thrombin generation. (50)

Roberts et. al. (47) reviewed studies analyzing bleeding and/or thrombosis incidences in patients with chronic liver diseases, acute liver failure or after liver transplantation. The conclusion was drawn that hemostasis is rebalanced and that both bleeding and thrombosis are common consequences in liver dysfunction, while in most cases the cause of bleeding is not due to dysfunction in coagulation itself. They postulate portal hypertension to be the reason for occurring bleedings.(47)

Mihăilă et al. (54) conducted a review on the connection between hypercoagulability and fibrogenesis in chronic liver diseases. It was conducted that liver disease results in a hypercoagulable state, favoring liver fibrogenesis due

to hepatic stellate cell (HSC) activation. Vice versa, anticoagulant therapy could be able to decrease the fibrogenesis rate.

Forkin et. al. (52) conclude in their study that taken derangements in both procoagulant and anticoagulant processes together, a balanced “normal” state can be seen at an early stage of liver disease. In the late state of liver disease, patients have an increased risk of bleeding and thrombosis, even possible at the same time. Cross et al. (37) emphasize that in many cases, liver disease leads to obstruction of bile ducts. In consequence, less or no bile is reaching the small intestine. As bile is necessary for absorbing vitamin K (fat), bile duct obstruction should lead to a decline in vitamin K dependent coagulation factors. However, as Cross et al. (37) mention in their review, Çakır et al. (55) could not demonstrate a decline in clotting activity in patients with obstructive jaundice and also no effect on coagulation parameters after drainage of obstruction, even though aPTT was prolongated. In contrast, the patients with obstructive jaundice showed a hypercoagulable state before and after drainage, as assessed by CCT, thromboelastography (TEG) and platelet function assay (PFA 100). In the majority, the underlying cause of obstruction was a malignant progress. This might lead to the assumption that malignancy is responsible for the hypercoagulable state, despite decreased coagulation factors and prolongated aPTT. However, it was shown that hypercoagulability correlated with increased bilirubin levels, which was also seen by Pihusch et al. (56) and Ben-Ari et al. (57) in obstructive jaundice due to benign biliary disease.

Tripodi et al. (58) draw a comparison between hemostasis in chronic liver disease (CLD) and in acute liver disease (ALD). In ALD, both anti- and procoagulants, with the exception of factor VIII and vWF (which are increased), and thrombocytes are severe decreased. Also in CLD, a variable decrease in both anti- and procoagulants is seen, with increase factor VIII and vWF. Thrombocytes are also decreased bit greater than in ALD. They see consumption in ALD and synthetic defects in CLD as the underlying cause of the deficiency in coagulation factors. (58)

In conclusion, liver disease does not lead to ‘auto-anticoagulation’ but instead presents a fragile but balanced hemostasis with a tendency to hypercoagulation (50) (49) and PT/INR are no suitable measurements to define coagulopathy in liver disease or as a guidance to apply transfusion of blood products (58) (49). Applying

FFP when actually not needed in terms of coagulation, could instead lead to bleeding due to an increased blood plasma volume and following hypertension (49). However, according to Rashidi-Alavijeh et al. (59) thromboelastometry is superior to CCT when evaluating the need for blood product transfusions but not in the case of prediction mortality in liver cirrhosis.

1.6. Aims and Objectives

Liver diseases are common in today's societies (liver cirrhosis is ranking 12th most common cause of death worldwide in 2016 (42)), potentially resulting in liver surgery or even requiring organ transplantation. With deceased organ donor transplants not being available to all patients in need, also living organ donors are in the focus (46).

The effects of liver surgery, especially on the CVS, are of great interest as they might result in a tremendous impact on a person's life quality. Perioperative risks and pathophysiological mechanisms, like coagulopathy, need to be identified to ensure adequate prophylaxis/treatment and patients' safety.

As described earlier, it is by now accepted and shown in several studies that in liver disease, especially in cirrhosis, a balanced but fragile hemostatic state prevails (49). One could argue that liver cirrhosis does not happen within a day and that liver function therefore should decline slowly, giving the opportunity for possible adaptions within the hemostatic system. This leads to the question, how hemostasis is influenced by the sudden decrease in functional liver parenchyma. Therefore, coagulation parameters measured in the perioperative setting to hepatectomy performed in healthy living liver donors is of special interest. But also, in liver disease patients undergoing hepatectomy it is of interest, how and to what extend the removal of diseased liver parenchyma affects hemostasis. Consequently, hepatectomy performed both in diseased and in healthy livers (living liver donors), is subject to this literature review.

It has been, for several reasons, decided to exclude recipients of liver transplant organs in this diploma thesis. First, and at least as the theoretical intention, these patients do not experience loss of liver parenchyma on a balance. Second, the surgery itself is of much greater extend, resulting in greater possible pathomechanisms to occur that might influence hemostasis. Third, the transplanted organ and the donor must be accounted for possible hemostatic diseases and health conditions.

2 Methods

For this diploma thesis, a systemically literature research on the subject of “effects of liver surgery on vascular health” in regards of coagulopathy was performed.

For the theoretical background, mainly textbooks (printed or online versions of books on physiology, pathophysiology, pathology, histology, biochemistry, pharmacology, and internal medicine) and some reviews were used.
Access to books online was gained through the library of the Medical University of Graz. PubMed and Web of Science were used to search for and to access relevant literature. Some full-text versions of articles and studies could be accessed for free, access to others was gained through the Medical University of Graz. To first gain an overview on the effects of liver diseases on hemostasis, PubMed and Web of Science were used to find studies, reviews, as well as seminars on that topic.
For the main part of this diploma thesis, a first search in PubMed was performed for literature published in 2015 or later, using MeSH terms “liver/surgery” AND “cardiovascular disease”. As the result were over 3630 entries, it was decided to narrow the cardiovascular effects of liver surgery down to changes in coagulation due to hepatectomy.
Words or MeSH major topics/MeSH terms used in this research were:
“liver”, “liver/surgery”, “liver resection”, “hepatectomy”, “living donors”, “hemostasis”, “blood coagulation”, “fibrinolysis”, “coagulopathy”, “thrombelastography”. These terms were used in different combinations, and by applying the Boolean operators “AND”, “OR”, “NOT”. To narrow the literature found down to only relevant studies, following exclusions were applied:

- No full text version
- No full access (for free or through the Medical University of Graz)
- Reviews
- Pediatric studies
- Animal studies
- No English or German language
- Year of publication before 2015
- Liver transplant recipients
- Studies comparing different medications

- No measurements of coagulation parameters performed

Furthermore, cited literature by the reviewed papers were looked at, to identify relevant publications not accessed in the initial search.

As illustrated in Figure 16, the literate review provided 15 relevant publications, after applying all defined criteria of exclusion.

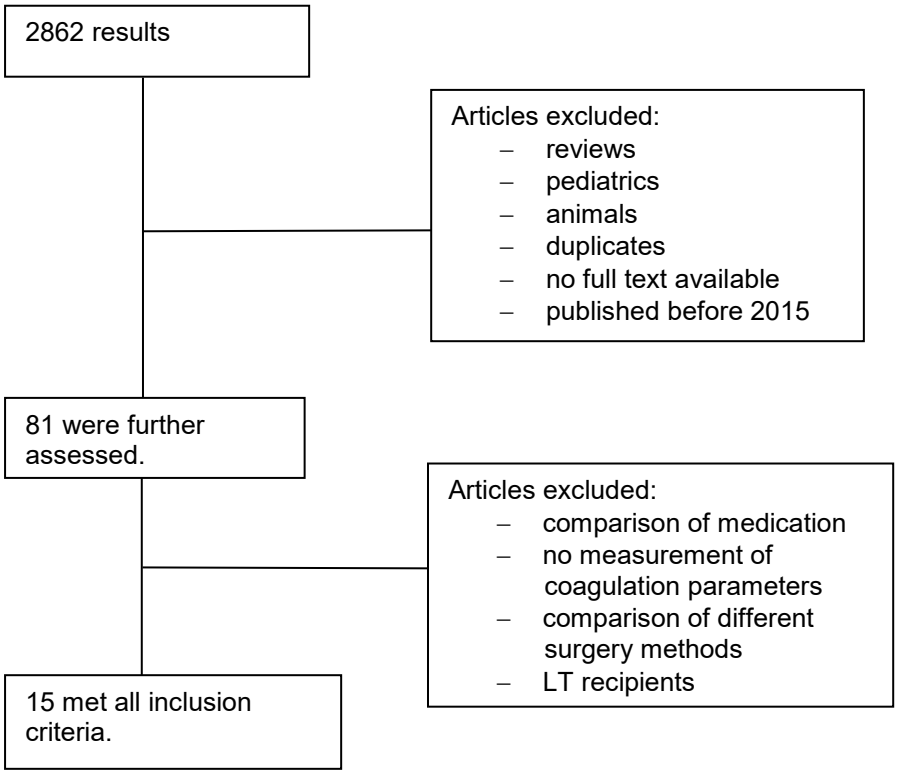

Figure 16: Flowchart of the selection process in the research using PubMed and Web of Science

For additional information or comparative purposes, some additional studies published before 2015 are referenced in the following.

3 Review of the Current Literature

A total of 15 studies were identified as relevant (as seen in Figure 16) and included in the following. Based on the underlying indication for performed hepatectomy, it was decided to categorize the relevant studies into two sections:

- **Section A** focuses on hepatectomy as a curative procedure in patients with malignant or benign liver lesions and diseases.
  Chapter 3.1. (Table 6) lists the twelve studies and their main characteristics. Followed by this, the studies' measurements are listed, grouped by CCT (Table 7), procoagulant factors (Table 8), anticoagulant and fibrinolytic factors (Table 9), and TGA and VET (Table 10).

- **Section B** focuses on living liver donors and accompanying changes in coagulation parameters.
  Chapter 4.2. (Table 11) lists the three studies and their main characteristics. Followed by that, Table 12 shows the results of CCT. As studies in Section B only performed CCT, there are no tables regarding procoagulants, anticoagulants and fibrinolytic factors, or TGA and VET.

In 3.3., the results of both Section A and Section B are analyzed and compared.

3.1 Section A: Hepatectomy as a Curative Procedure

Table 6: Section A - Hepatectomy performed as a curative procedure

Author	Year of publication	Study cohort	Study design	Parameters measured	Outcome/Conclusion
Dumitrescu et al. (60)	2015	16 liver resections – 8 hemihepatectomies – 8 extended hemihepatectomies	Descriptive pilot study	– CCT: INR, aPTT, platelet count, fibrinogen, – d-dimers – VET: ROTEM	CCT: abnormalities in coagulation ROTEM®: **balanced coagulation**
Gordon et al. (61)	2015	77 hepatectomies. Indications: - 14 benign lesions - 63 malignant lesions: - 25 primary lesions - 38 metastatic lesions	Retrospective study	VET: TEG (R time, K time, alpha angle)	**Relative hypercoagulable** state post-surgery. TEG showed no differences between benign and malignant groups.
Potze et al. (62)	2015	17 partial hepatectomies – 15 right and 2 extended right hemihepatectomy – 53% metastasis from colorectal cancer – 12% each: HCC and CCC	Prospective study	– CCT (PT, aPTT), – factor VIII & II, AT, protein C, – TGA (Calibrated Automated Thrombography® CAT)	**Hypercoagulability** after hemihepatectomy as potentially caused by decrease in protein C and S, and AT and by increase in factor VIII
Kleiss et al.(63)	2016	10 pancreas resections 24 healthy controls	Retrospective study	Fibrinolytic proteins: PAI-1, plasminogen, α2-antiplasmin, TAFI	**Fibrinolytic potential decreased** post-surgery, normalized on POD1, hypofibrinolytic state on POD3. Recovery slower in hepatectomy.

Mallett et al. (64)	2016	45 major hepatic resections (≥30% volume resection) – 60% right and extended right hemihepatectomy – 22% left and extended left hemihepatectomy – 18% multiple non-contiguous segmentectomy –	Prospective study	– CCT (INR, PT, aPTT, fibrinogen), – factors II, VII, VIII, X, and XI, vWF, protein C and S, AT, d-dimers, – TGA – VET: ROTEM	**Prothrombotic** environment in the early postoperative period.
Karanicolas et al. (31)	2016	18 major liver resections – 16 metastases from CRC – 1 HCC – 1 CCC – 6 controls with no TXA – 12 with TXA	Prospective study	– CCT: INR, PTT, fibrinogen – PAP complex – VET: TEG – TXA concentration	**No evidence in TEG of hyperfibrinolysis** after major hepatectomy. TXA was not shown to influence systemic fibrinolysis.
Groeneveld et al.(23)	2016	41 probands – 17 hepatectomies: 15 right, and 2 left hemihepatectomies – 10 PPPD – 24 healthy controls	Prospective study	vWF ADAMTS13	Imbalance between vWF and ADAMTS13 levels and activity after surgery: – Due to **increased vWF** activity to antigen ratio and increase in HMW vWF multimers – More pronounced after hepatectomy (for up to 30 days).

Singh et al. (65)	2017	86 major hepatectomies - 40 HCC - 17 hilar cholangiocarcinoma - 57% resection of four segments	Retrospective study	CCT: Platelet count, INR	45.34% derangement in coagulation profile on POD1: **Platelet count** was significant lower, **INR** significant higher
Le et al. (33)	2016	87 hepato-pancreato-biliary (HPB) surgeries, of which: - 35 minor hepatectomies - 15 major hepatectomies - 37 PPPD - 79 malignant, 8 benign	Retrospective study	VET: TEG	No significant trend between pre- and postoperative TEG parameters in hepatectomies. 46.6% of all patients undergoing HPB were **hypercoagulable**.
Jacquenod et al.(66)	2017	759 patients after liver surgery	Retrospective study	Platelet count, INR	**Coagulopathy** observed in 53,5% (hypocoagulable)
Tanner et al. (67)	2017	33 hepatectomies - 11 cirrhotic livers - 22 non cirrhotic livers (7 benign and 26 malignant lesions)	Prospective study	– CCT: INR, PT, PTT – VET: TEG	**Similar coagulation profile** after hepatectomy in non-cirrhosis and cirrhosis. **Hypercoagulable** profile in many probands.
Oo et al. (68)	2020	41 major hepatectomy (removal of ≥ 3 segments) - 90.3%: malignancy - 70.7%: metastases from CRC	Prospective study	– CCT: INR, PT, aPTT, fibrinogen – vWF-Ag, clotting factors, d-dimers – VET: TEG	**INR elevated** post-surgery **TEG parameters remained normal** or supranormal

3.1.1 Conventional Coagulation Tests

Table 7: Section A - CCT

Author	INR	PT (Quick)	aPTT	Fibrinogen	Platelet count
Dumitrescu et al. (60)	**Increase** postop with peak on POD1, return to normal range (decreasing from peak but still higher than pre-surgery) on POD4 and 7		**Increase** on POD1	**POD1: decreased** compared with preoperative values POD4&7: slightly increased from POD1, still below baseline	**POD1: decreased** compared with preoperative values POD4: increased from POD1 level, but no baseline levels reached **POD7: exceeded baseline levels**
Gordon et al. (61)	-	-	-	-	-
Potze et al. (62)	-	Progressively **increased**: peak on POD 1 (18.3 s). Decreased after POD1, reached baseline on POD7.	Slight **decrease**	-	-
Kleiss et al. (63)	-	-	-	-	-
Mallett et al. (64)	**Increase** from end of surgery, peak day 1 (30% INR ≥2,0). Day 5: all but one had INR≤1,5	**Increased** with peak at POD1. On POD5 within range again	Remained within **normal range**	**Decrease** with nadir by the end of surgery. **Increased above baseline from POD2**	**Decreased** after surgery with nadir on POD1
Karanicolas et al. (31)	**Increased** significantly by the end of parenchymal transection. Continued to rise slightly by the morning of POD1	-	-	**Decreased** steadily during operation, then increasing by the morning of POD1. No return to baseline.	-

Singh et al. (65)	Significantly **higher** on POD1, peak on POD3&4, decrease from POD4 on	-	-	-	(vWF dependent platelet adhesion increased)
Le et al. (33)	-	-	-	-	Significant **decrease** on POD1-POD5
Groeneveld et al. (23)	-	-	-	-	-
Jacquenod et al. (66)	Maximal **derangement** on POD 1. 17% still presented an INR ≥1,5 and/or platelet count <80G/L on PODs 4-5	-	-	-	**Decrease** with nadir on POD2 and 3
Tanner et al. (67)	Significantly **higher** on POD1 and 3	Significantly **higher** POD1 and 3	-	-	Significantly **lower** on POD1 and POD3 than preoperative
Oo et al. (68)	Within **normal range** at end of surgery. Significantly **elevated** on POD1. INR >1,5 in 17,1% on POD1 and 2,4% on POD3	-	Within **normal range** at end of surgery. **Increase** on POD1 and continuous rise on POD3	**Normal range** at baseline and during surgery. **Raise on POD1 and POD3**	

3.1.2 Procoagulant Factors

Table 8: Section A - Procoagulant factors

Author	Factor II	Factor V	Factor VII	Factor VIII	Factor X	Factor IX	vWF
Dumitrescu et al. (60)	-	-	-	-	-	-	-
Gordon et al. (61)	-	-	-	-	-	-	-
Potze et al. (62)	**Decrease** port-surgery. Baseline reached again on POD30	-	-	Higher levels pre-surgery than in controls. **Increase post-surgery** with peak on POD5. Decreased after POD7, but remained higher than in controls	-	-	-
Kleiss et al. (63)	-	-	-	-	-	-	-
Mallett et al. (64)	**Decrease** postoperative with nadir on POD1. POD5: returned to baseline	**Decrease** postoperative with nadir on POD1. POD5: returned to baseline	**Decrease** postoperatively with nadir on POD1. POD5: returned to baseline	Steady **rise** from POD1	**Decrease** postoperative with nadir on POD1. Return to baseline on POD5.	-	Steady **rise** from POD1
Karanicolas et al. (31)	-	-	-	-	-	-	-
Groeneveld et al. (23)	-	-	-	-	-	-	**Increased** vWF functional activity, and vWF-dependent platelet adhesion in vitro.
Singh et al. (65)	-	-	-	-	-	-	-

Le et. al. (33)	-	-	-	-	-	-	-
Jacquenod et al. (66)	Remained within **normal** range	-	Transient **reduction**	-	Remained within **normal** range	-	-
Tanner et al. (67)	-	-	-	-	-	-	-
Oo et al. (68)	**No change** from baseline		**Decreased** from baseline on POD1 but levels began to increase on POD3	VIIIc: **No change** from baseline	**No change** from baseline	**No change** from baseline	vWF-Ag: showed marginal significant change from baseline but remained within **normal range** at all time points

3.1.3 Anticoagulant Factors

Table 9: Section A - Anticoagulant and fibrinolytic factors

Author	Protein C	Protein S	Antithrombin	Fibrinolytic parameters	ADAMTS13	D-dimers
Dumitrescu et al. (60)	**Decreased** compared with preoperative values. Nadir POD4	**Decreased** compared with preoperative values. Nadir POD1	**Decreased** compared with preoperative values. Nadir POD4	-	-	**Increase** on POD1. No return to baseline on POD7
Gordon et al. (61)	-	-	-	-	-	-
Potze et al. (62)	**Decreased** until POD1. Back to baseline on POD30	-	**Decreased** from end of surgery until end of POD3. Back to baseline on POD30	-	-	-
Kleiss et al. (63)	-	-	-	**Decreased fibrinolytic potential** post-surgery (with	-	-

				increased plasma levels of PAI-1), normalized on POD1. Again hypofibrinolytic state on POD3. **Decrease in plasminogen, α2-antiplasmin, TAFI**.		
Mallett et al. (64)	**Decrease** post-surgery. Nadir on POD1. POD5: remained low	**Decrease** post-surgery. Nadir POD1. POD2: baseline. POD5: higher than preoperative	**Decrease** post-surgery. Nadir on POD1. POD5: remained low	-	-	**Increased** post-surgery. Remained increased on POD5
Karanicolas et al. (31)	-	-	-	**Transient fibrinolytic activity** (TEG: FIBTEM): **Increasing PAP activity** after parenchymal transection. Decreased to baseline by POD1.	-	-
Groeneveld et al. (23)	-	-	-	-	ADAMTS13 activity **decreased**	-
Singh et al. (65)	-	-	-	-	-	-
Le et al. (33)	-	-	-	-	-	-
Jacquenod et al. (66)	-	-	-	-	-	-
Tanner et al. (67)	-	-	-	-	-	-
Oo et al. (68)	-	-	-	-	-	**Normal range** at end of surgery.

3.1.4 Thrombin Generation Assay and Viscoelastic Tests

Table 10: Section A – TGA and VET

Author	Thrombin Generation Assay (TGA)	Viscoelastic Tests (VET)	
		ROTEM	TEG
Dumitrescu et al. (60)	-	Median values stayed within normal: – POD1: increase in CFT-INTEM, CFT-EXTEM – POD1: decrease in MCF-INTEM, MCF-EXTEM, MCF-FIBTEM – POD1: no change in CT-INTEM, CT-EXTEM	-
Gordon et al. (61)	-	-	Hypercoagulable state – R-time: decreased at least up to POD5 – Mean values for R time, K time, alpha angle, MA within normal range
Potze et al. (62)	Hypercoagulable state post-hepatectomy: – ETP decreased until POD7. Baseline on POD30 – TG with TM: increased – TG without TM decreased – TM-SR increased – Peak TG levels and lag-time were close to baseline – Peak TG with TM: gradually increased – Velocity index increased until POD30, peak POD3	-	-
Kleiss et al. (63)	-	-	-
Mallett et al. (64)	Remained within normal range But ETP higher on POD1 than pre-operative	Overall: Remained within normal range (EXTEM-MCF,-CT, INTEM, FIBTEM)	-

Karanicolas et al. (31)	-		Stayed within normal range: – EXTEM: CT, alpha angle, MCF, ML, lysis index after 30min – FIBTEM: MCF ML, lysis index after 30min
Groeneveld et al. (23)	-	-	-
Singh et al. (65)	-	-	-
Le et al. (33)	-		No significant changes between pre- and post-surgery values. TEG results: 46.6% of patients were hypercoagulable perioperative
Jacquenod et al. (66)	-	-	-
Tanner et al. (67)	-		Hypercoagulable state in up to 64% at some point post-surgery (Parameters: MA, R time, K time, alpha angle, percent lysis at 30min, coagulation index)**
Oo et al. (68)	-		Median parameters (MA, alpha angle, K time, R time) stayed within normal range at all times

*TM-SR: Thrombomodulin sensitivity ratio. TEG-MA: TEG-Maximum Amplitude. CT: Clotting time. MCF: maximum clot firmness. ML: maximum lysis
** Data was not available for free

3.2 Section B: Hepatectomy in Healthy Living Liver Donors

Table 11: Section B – Hepatectomy performed in living donors

Author	Year of publication	Cohort	Study design	Coagulation parameters	Outcome
Karna et al. (69)	2015	100 living liver donors - 86 right lobe hepatectomy - 14 left lobe hepatectomy	Retrospective study	INR, platelet count Coagulopathy defined as INR >1.5 or PC <1x10^5/mm^3	Coagulopathy observed in 84%
Aktas et al. (46)	2015	46 living liver donors undergoing hepatectomy	Retrospective study	PT, INR, platelet count Coagulopathy defined as PT>15sec. or PC <80000/mm^3 on POD3	24 donors with coagulopathy 22 donors without coagulopathy Lower remnant liver volume = significant risk factor for coagulopathy
Berglund et al. (70)	2018	176 living liver donors -154 right hepatectomy - 4 left hepatectomy -18 left lateral segmentectomy	Retrospective study	INR	INR >2 post-surgery was associated with a higher risk of complications

3.2.1 Conventional Coagulation Tests

Table 12: Section B – Conventional Coagulation Tests (CCT)

	INR	PT	aPTT	Fibrinogen	Platelet count
Karna et al. (69)	Rise in all. Peak at POD2. On POD5 mean INR = 1.3	-	-	-	Decreased in all, with nadir on POD3
Aktas et al. (46)	-	POD3: >0.15sec in 52,17%	-	-	None with PC <80,000 on POD3
Berglund et al. (70)	Increase with peak on POD2. None had INR>2 at 1 week post-donation. Peak INR value was higher among right-lobe donors than left-lobe donors	-	-	-	-

3.3 Analysis

3.3.1 Conventional Coagulation Tests

Section A

Table 7 shows results of CCT measured by studies of Section A. It is to note that not all studies performed all tests, and Gordon et al. and Groeneveld et al. did not perform CCT at all. Kleiss et al. and Potze et al. refer to the same study cohort, which explains the lack of measurements by Kleiss et al.

INR or PT were increased post-surgery in all studies that performed measurement ((60), (62), (64), (31), (65), (66), (67), (68)) while Oo et al. still measured a normal range at the end of surgery, followed by an increase thereafter. The aPTT was measured either within normal range post-surgery (64) or increased on POD1 ((60), (68)). While Oo et al. measured an elevated aPTT on POD1, it was still within normal range immediately at the end of surgery (68).

During surgery or by the end of surgery, fibrinogen levels were decreased ((60), (64), (31)) or in normal range (68). In the postoperative phase, fibrinogen levels increased ((64),(68)). Platelet count decreased post-surgery ((60), (64), (65), (66), (67)). Most parameters showing decreased levels, were again increasing in the postoperative phase towards baseline levels.

Section B

All studies included in section B measured INR or PT (see Table 12). In all, an increase could be noted, with a peak around POD2 (69,70) or POD3 (46). Platelet count was shown to be decreased with the nadir on POD3 by Karna et al. (69), while Aktas et al. only refered to no patients showing a platelet count below 80,000 on POD3 (46).

3.3.2 Procoagulants

Section A

Table 8 shows measured procoagulant factors by studies of section A. While some studies only measured some of the shown procoagulant factors, Gordon et al., (Kleiss et al. - see Potze et al.), and Le et al. measured none of them. This narrows the validity of these parameters within Section A.

Postoperatively, factor II decreased ((62), (64)) or stayed within the normal range ((66), (68)). Factor V decreased (64), as well as factor VII ((64), (66), (68)). Factor X decreased as well (64) or stayed within normal range or at baseline ((66), (68)). Factor IX showed no change from baseline (68). Levels of vWF (64) and its activity (23) increased, or (as seen by vWF antigen) stayed within normal range (68). In contrast, factor VIII was shown to be increased ((62), (64)), or showed no change form baseline levels (68).

Section B

None of the studies in Section B performed measurements of procoagulant factors in living liver donors.

3.3.3 Anticoagulants

Section A

Anticoagulant factors measured by studies of section A are shown in Table 9. There was no study measuring all the listed factors. Gordon et al., Singh et al., Le et al., Jacquenod et al., and Tanner et al. did not measure any of the listed parameters. This results in only a few measurements of anticoagulant factors, providing a trend, but no significancy.

Protein C was shown to be decreased post-surgery ((60), (62), (64)), as well as Protein S ((60), (64)), antithrombin ((60), (62), (64)), and activity level of ADAMTS-13 (23). As for fibrinolytic parameters, Kleiss et al. showed two waves of a hypofibrinolytic state, with the first post-surgery and the second on post-operative day three (POD3). They showed a decrease in PAI-1 levels post-surgery and decreased levels in plasminogen, α2-antiplasmin, and TAFI (63). Karanicolas et al. showed an increased activity of PAP that reached baseline levels again on POD1. Groeneveld et al. (23) see the reason for the decrease in ADAMTS-13 activity in the increase of high molecular weight (HMW) vWF multimers, which has been shown to be associated with a decrease in ADMATS-13 levels (either due to ‘exhausting’ by the increased substrate, or by consumption (71)). D-Dimers were shown increased post-surgery ((60), (64) or within normal range at end of surgery (68).

Section B

None of the studies in Section B performed measurements of anticoagulant factors such as protein C and S, AT or fibrinolytic parameters in living liver donors.

3.3.4 Fibrinolysis

An increased risk for thromboembolism or bleeding events is not only given due to disturbed coagulation but also due to disturbed fibrinolysis. Compared to studies looking at perioperative coagulation, there is little systemically studying of perioperative fibrinolysis.

Section A

Kleiss et. al (63) studied parameters of fibrinolysis in patients undergoing hepatectomy or PPPD. They were able to show that after both surgeries a state of hypofibrinolysis, as detected by an increase in clot lysis time (CLT), resulted. Thereby, hypofibrinolysis occurred in two waves, with the first detected at the end of surgery, and the second on postoperative day three (POD3). After POD3, the hypofibrinolytic state started to return slowly to normal levels on POD30. They explained the underlying pathomechanism for the first wave of increased CLT due to an increased level of plasma plasminogen-activator-inhibitor type-1 (PAI-1). They refered to Kassis and Podor (72), who were able to show that PAI-1 synthesis and secretion by endothelial cells was activated postoperatively. They further confirmed higher levels in PAI-1 to be linked to ‘postoperative fibrinolytic shutdown’. As for the second wave of hypofibrinolysis, Kleiss at al. (63) see a more complex pathomechanism responsible: postoperative decreases in plasminogen, α2-antiplasmin, TAFI, and prothrombin levels were seen and postulated to be caused by first, consumption due to activated fibrinolysis, and second, due to hemodilution. As reaching of baseline levels postoperatively was seen to be different for each protein, a state in which antifibrinolytic proteins outweigh the profibrinolytic ones, hence causing a hypofibrinolytic state on POD3-7, was occurring. With less liver parenchyma or a decreased liver function, not only the production of certain proteins is impaired but also their clearance. This was held responsible for observed increase in levels of PAI-1 post hepatectomy. Additionally, it was thought that a greater surgical trauma to the liver releases more PAI-1, that is present in great amounts in the liver and is mainly synthesized by endothelial cells.

Karanicolas et al. (31) measured thromboelastographic parameters in patients undergoing major hepatectomy and analyzed them in regards of tranexamic acid application. They could not demonstrate hyperfibrinolysis in these patients using TEG analysis. They were able to show that tranexamic acid had no effect on systemic fibrinolysis. However, they stress that notable changes in TEG regarding hyperfibrinolysis would require massive near-fatal trauma. Measurements of the plasmin-antiplasmin (PAP) complex levels, a complex consisting of plasmin and α2-antiplasmin, was used as a marker for the systemic fibrinolytic activity, as PAP acts as an indicator for recently occurred fibrinolysis. PAP levels increased modestly during hepatectomy and decreased by POD1 in all patients, disregarding tranexamic acid application. Fibrinogen levels decreased during surgery and increased on POD1, without reaching baseline levels again in time of observation. However, Karanicolas et al. stress, that levels of fibrinogen were not sensitive enough to be used for elevating fibrinolysis during hepatectomy.

Concluding, Kleiss et al. (63) showed two waves of decreased fibrinolytic potential, while Karanicolas et al. (31) showed a transient fibrinolytic activity during surgery by measuring PAP levels. Looking at these two studies, it is of notion that Kleiss et al. and Karanicolas et al. used different parameters, assessing fibrinolytic activity differently. Therefore, these findings might not disagree.

Section B

None of the studies in Section B performed measurements of fibrinolytic parameters in living liver donors.

3.3.5 TGA and VET in comparison to CCT

Section A

In the two studies of section A that performed TGA (see Table 10), values showed either a hypercoagulable state (62) or stayed within the normal range (64) post-surgery. This is in both cases contrary to the measured increases in the INR.

Median measurement values obtained by ROTEM stayed within normal range overall (60,64). TEG parameters revealed either a hypercoagulable state post-surgery (61,67) or stayed within the normal range overall (31,68). Le et al. could

not show a significant change from pre- to post-surgery TEG values, but 46.6% of patients were identified as hypercoagulable perioperatively by TEG (33).

Dumitrescu et al. (60) measured PT, INR, aPTT, d-dimers, fibrinogen, platelet count, ATIII, protein C and S, and ROTEM parameters in patients undergoing major hepatectomy. While the ROTEM parameters, with exception for some individual values of CT-EXTEM and FIBTEM being elevated on POD4 and 7, stayed within the normal range, INR and aPTT increased postoperatively.
INTEM and EXTEM did not show a change in parameters, as it would have been suspected by the decrease in fibrinogen and procoagulant factor levels. They see the reason for normal ranged ROTEM parameters on POD1 in first, the simultaneously decrease in both pro- and anticoagulant factors, and second, in a still high enough platelet count to ensure sufficient clot strength. Furthermore, they stress the role of erythrocytes in coagulation and to the results obtained by ROTEM.

Gordon et al. (61) measured TEG values in patients undergoing hepatectomy due to either benign or malignant liver lesions. They were able to show that while overall TEG parameters showed no difference between the two groups, the post-operative parameters indicated a relative hypercoagulable state (decrease in R-time up to POD5). They therefore postulate that the relative hypercoagulable state was not caused by malignancy but by postoperative coagulopathy. Without presenting their measurements of the INR, they stated an elevated INR in many patients at the end of surgery. This is especially notable, considering malignancy usually acts promoting coagulation and is reported to increase the risk for VTE by seven (37).

Potze et. al (62) compared thrombin generation profiles with CCT in patients undergoing right hepatectomy or pancreatic resection. They found a hypercoagulable state post right hemihepatectomy identified by ETP but not by CCT. The latter, in contrast, were prolonged. By performing TGA with and without thrombomodulin (TM), they could show a resistance to TM post-hepatectomy. They explain this due to the decrease in protein C and the increase in factor VIII.
As TM activates protein C, TGA parameters decreased after adding TM in controls, but in protein C deficiency TGA would only slightly decrease. Additionally,

AT could be shown to be increased. Taking these changes together, normal or elevated TGA parameters could be measured post-hepatectomy when TM was added. This explains a normal or hypercoagulable state identified by TGA, even with a measured decrease in procoagulant factors.

Mallett et al. (64) analyzed thrombin generation parameters, thromboelastometry, CCT (PT, INR, aPTT), clotting factors, vWF, protein C and S, fibrinogen, d-dimers, and ATIII in patients undergoing major hepatectomy. They found a disbalance between anti- and procoagulant factors, in favor of a prothrombotic state early after surgery. While INR was shown to be prolonged in the early postoperative state, thromboelastometry and thrombin generation parameters showed normal levels.

Karanicolas et al. (31) showed that after hepatectomy, TEG parameters stayed within the normal range, while INR increased significantly after surgery and in the early postoperative days.

Le et al. (33) analyzed perioperative TEG parameters in patients undergoing hepato-pancreato-biliary surgery and found no significant trend between pre- and postoperative TEG parameters in hepatectomies. Three patients undergoing major hepatectomy and eleven undergoing minor hepatectomy showed perioperative changes in TEG, whereby in the firsts, normal parameters changed towards hypocoagulable state in 67.7% and in the latter, normal parameters changed towards a hypercoagulable state in 36.4%. However, perioperative changes in TEG parameters were not found significant in the hepatectomy group. Also, they have not found an association between neither the preoperative TEG parameters nor the postoperative TEG parameters and the INR. Chemoprophylaxis of venous thromboembolism (VTE) was administered to 95.4% of all patients preoperatively and to 100% of all patients postoperatively.

Tanner et al. (67) showed that patients undergoing hepatectomy presented a hypercoagulable state as identified by TEG, whereas CCT were prolonged and platelet count declined. This observation was made whether the liver was in a cirrhotic state or not.

Oo et al. (68) assessed CCT (INR, PT, aPTT), procoagulant activity of phospholipids and clotting factors, fibrinogen, d-dimer vWF antigen, and TEG parameters in patients undergoing hepatectomy. While TEG parameters stayed within the normal range, CCT showed a prolongation.

Section B

Among the considered studies in Section B, there is none performing VET analysis perioperatively to hepatectomy.

3.3.6 Correlations with Remnant Liver

It is of question, whether measured changes in hemostatic parameters were correlating with the percentage of lost liver parenchyma, the percentage of remnant liver, or with the surgical procedure itself. This will help in the question how much liver tissue is safe to resect, while still guaranteeing adequate hemostasis. The usual surgical guide is to leave a patient with at least 30% of liver function post hepatectomy (45).

Section A

Dumitrescu et al. (60) compared changes in coagulopathy in patients undergoing hemihepatectomy with those undergoing extended hemihepatectomy. They were able to show that fibrinogen plasma concentrations, as well as MCF-FIBTEM were lower in extended hemihepatectomy than in hemihepatectomy. As fibrinogen is an acute phase protein mainly synthesized by the liver, they considered a greater remnant liver volume contributing to a potential overall hypercoagulable state. However, other measurements were not analyzed by Dumitrescu et al. regarding an association towards remnant liver volume.

Kleiss et al. (63) postulate, that along with greater surgical trauma, more tissue damage to the liver occurred, leading to a greater release of PAI-1. Following, fibrinolysis would be more disturbed after major hepatectomy than after minor one.

Jacquenod et al. (66) showed that patients undergoing major hepatectomy, with resection of a minimum of 3 liver segments, had a higher incidence of coagulopathy than patients undergoing minor hepatectomy. Further analyses confirmed the association of major hepatectomy and coagulopathy to be

significant. They see the reason for elevation in INR post hepatectomy in a reduction of hepatocytes and the following reduced capacity in production of clotting factors.

Section B

Karna et al. (69) were able to identify the percentage of remnant liver volume as an independent predictor of coagulopathy following hepatectomy, with right lobe hepatectomy showing a greater risk for coagulopathy than left lobe hepatectomy.

Aktas et al. (46) found an association between remnant liver volume, remnant liver volume percent, remnant liver volume to body weight ratio and coagulopathy after hepatectomy in living liver donors. Further analysis identified remnant liver volume percent underneath 40.5% to be the only statistically significant independent risk factor for coagulopathy. Additionally, they conclude that complications, mortality, and overall negative effects on quality of life, were lower among left lateral lobe hepatectomies due to a greater remnant liver volume percent. Aktas et al. imply to question the postulated safe minimal remnant liver volume percent in donors of 30% (73) as they found already a remnant liver volume <40.5% to be an independent risk factor regarding coagulopathy in healthy living liver donors.

Berglund et al. (70) could show a significant association between lower remnant liver volume and complications grade 3 after hepatectomy.

In conclusion, it can be said, that the more liver parenchyma is lost due to hepatectomy, the greater the risk for coagulopathy to occur. However, the question prevails, how much actually functional liver parenchyma is lost when resecting diseased liver or lesion.

3.3.7 Correlation to Thrombosis/ Thromboembolic Events or Bleedings

Disturbed coagulation and/or fibrinolysis are risk factors for either bleedings or thrombi, going along with higher risks for thromboembolic events, to occur. Therefore, the question that arises is, whether the postoperative hypercoagulable state, as identified by VET, leads to clinically relevant thromboembolic events or if

elevated CCT actually indicated hypocoagulability leading to postoperative bleedings.

Section A

Dumitrescu et al. (60) reported that 12,5% (n=2, both right hepatectomy) of all patients were diagnosed with PAE on POD5 and POD8. Retrospectively, some of the ROTEM parameters revealed hypercoagulation before the thrombotic event. They did not note any clinically significant signs of postoperative bleeding.

Gordon et al. (61) observed no DVT of lower extremities or PE in patients during their hospital stay. 2 out of 77 (2.6%) patients developed internal jugular vein thrombosis. While these 2 patients had hepatic metastases from CRC, they see the epidural catheter induced trauma as the underlying cause for thrombosis and not malignancy itself. There is no mention regarding postoperative bleeding.

Mallett et al (64) report of 4.67% (n=2) of patients who showed venous thromboembolism, with one on POD3 and one on POD4. The first one´s INR on POD2 was 1.7 and the second one´s INR on POD2 was 2.3. 2.33% (n=1) of patients developed a deep vein thrombosis (DVT) on POD14 and started medical thromboprophylaxis on POD5. All patients were reported to have additional risk factors for thrombosis: elderly patients, more extend resections, and delay in initiation of medical thromboprophylaxis. They do not mention whether postoperative bleeding occurred.

Karanicolas et al. (31) reported no thromboembolic events during the study period. One patient received a blood transfusion, but no patients needed to be readmitted due to bleeding.

Le et al. (33) observed no VTE in the hepatectomy group within 30 days post-surgery. There was one patient requiring blood transfusion within 30 days after surgery. However, this was due to septic shock following colonic leak.

While not mentioning any bleeding events or incidence of thrombosis within the cohort, Groeneveld et al. (23) point out that the observed imbalance between vWF and ADAMTS13 may cause thrombi within microvascular vessels. These might not

be clinically relevant or detectable but could lead to occlusion of microvasculature and organ damage and dysfunction.

Also, Oo et al (68) reported no thromboembolic events up until POD30. However, 4.88% (n=2) of patients showed pulmonary embolism three and six month after surgery, even with applied thromboprophylaxis started on the day of surgery. Of 17% of patients with an elevated INR>1.5, one patient required blood transfusion for decreased hemoglobin, while the criteria for post-hepatectomy hemorrhage were not met. One patient with normal coagulation parameters showed grade A post-hepatectomy hemorrhage.

Tanner et al. (67), Jacquenod et al. (66), and Potze et al. (62) all do not mention whether bleeding events occurred in their studies.

On an additional note from older study not included in Section A: Ejaz et al. (74) conducted a study (published in 2014) with 599 patients undergoing hepatectomy, mainly for malignancy (90.8%), in order to identify incidence and risk factors of following VTE. They were able to show that almost 14,29% of patients undergoing extended hepatectomy showed venous thromboembolism within three months post-surgery. Notably, patients with an elevated INR showed a higher incidence of VTE after hepatectomy even with prophylaxis applied in most cases. Furthermore, no significant difference was seen between hepatectomy due to malignancy or a benign lesion.

Section B

Neither Karna et al. (69), nor Aktas et al. (46) mentioned whether thrombosis or thromboembolic events occurred within their cohorts.

While Karna et al. did not mention whether bleeding events occurred in the cohort, Aktas et al. mentioned two patients with significant intraoperative bleeding due to sliding of a clamp.

Berglund et al. (70) did not report any PE, but after right lobe hepatectomy, 0.6% patients showed DVT (n=1), while none after left lobe hepatectomy until POD90. In 1.9% (n=3) postoperative abdominal bleeding occurred.

On an additional note from an older study not included in section B: A retrospective cohort study performed by Takagi et al. (75) short- and long-term outcomes in living liver donors were analyzed. Among 408 patients, complications occurred in 40.4% post-hepatectomy. Thromboembolic complications (including hepatic vein thrombosis, deep vein thrombosis, and pulmonary embolism) occurred in 2.2% (n=9), with the majority of them (n=6) developing after right lobe hepatectomy.

3.3.8 Comparison to General Abdominal Surgery

Not only hepatectomy, but in general major surgeries can promote a prothrombotic state after surgery. This is postulated to be, among other possible factors, caused by exposure of TF due to damaged tissue and vessels, by an altered blood flow, by inflammatory processes and by a weakened fibrinolysis (76). While major surgeries are well known to be a risk factor for thrombosis/thromboembolism, there is often delay in thromboprophylaxis in the setting of hepatectomy due to an elevated INR (62). The question arises, whether patients undergoing hepatectomy are at the same or a different risk than patients undergoing other (major abdominal) surgeries.

Potze et al. (62) compared changes in hemostatic parameters, especially thrombin generation (TG), after right hemi-hepatectomy with those after pylorus-preserving pancreaticoduodenectomy (PPPD). They were able to show that the endogenous thrombin potential (ETP) was decreased in the first seven days past right hemi-hepatectomy and past PPPD, whereas the decrease was greater after the latter. After adding thrombomodulin (TM), TG was increased after hemi-hepatectomy and only slightly increased after PPPD. It could be shown that TM was not as sufficient in terms of thrombin generation regulation post hemi-hepatectomy than post PPPD. They explained this by decreased levels of protein C and AT, in addition to elevated levels of factor VIII. As they measured a decrease of factor II, AT and protein C after PPPD as well, they see the underlying causes also in general damage caused by surgery and hemodilution (62). However, the effects seen in TGA differed between the two surgeries, with a greater decrease of protein C and AT after hepatectomy, putting loss of liver tissue as the main reason for observed hypercoagulability.

Kleiss et al. (63) showed that levels of plasminogen decreased after both partial hepatectomy and PPPD but recovered much slower after partial hepatectomy than after PPPD. The same was shown for TAFI levels as they decreased in both groups and showed a slower recovery after partial hepatectomy than after PPPD. Also, α2-antiplasmin levels decreased in both groups, whereas they reached baseline levels again earlier than plasminogen levels. The levels of PAI-1 increased in both groups at the end of surgery, with being significantly higher after partial hepatectomy, and decreased again on POD1. In patients undergoing partial hepatectomy, PAI-1 levels increased again on POD3 and thereafter reached baseline on POD30. In patients undergoing PPPD baseline levels were reached on POD1. Kleiss et al. see these changes associated by the impaired liver function following hepatectomy and not just abdominal surgery in general, as the observed changes recovered much faster after PPPD.

Groeneveld et al. (23) compared plasma levels and activity levels of vWF, vWF-dependent platelet adhesion, as well as plasma levels of ADAMTS13 after partial hepatectomy with those after PPPD. They could show, that both postoperative phases went along with imbalances in the vWF-ADAMTS13 axis. However, these changes were much greater after partial hepatectomy than after PPPD, indicating that this is specific to liver surgery and not abdominal surgery in general. They see this imbalance caused in an increase of the vWF-activity/vWF-antigen ratio and in an increase in HMW vWF multimers. Groeneveld et al. noted a more distinct increase of vWF after PPPD than after hepatectomy and gave two possible reasons for that. First, more endothelial damage would lead to an increase of vWF in PPPD. Second, in PPPD the liver's clearance of vWF worked better than after hepatectomy.

In contrast, Le et al. (33) showed, that while the majority of patients showed normal TEG parameters both after hepatectomy and pancreatectomy, more hypercoagulable states were seen after pancreatic resection than after hepatectomy.

Additional notes of interest from older studies not included in section A or B

In an earlier study, published in 2001, Mahla et al. (77) analyzed thromboelastographic changes after major abdominal surgery. They could show a hypercoagulable state for at least seven days postoperatively. The investigated surgeries included gastrectomies, colonic resections and Whipple procedures. Fibrinogen was significantly increased after surgery but was not held responsible for the hypercoagulable state. Instead, a considerable platelet activity was accounted for postoperative hypercoagulability.

De Pietri et al. (78) showed in a study published in 2010 that according to thromboelastography tracings, a normal coagulation state prevails after liver surgery and a temporary hypocoagulable state after pancreatic surgery. They see the pathomechanism for the latter in the release of pancreatic enzymes (like trypsin) due to surgical organ damage and an acute pancreatitis like reaction followed by an upregulated consumption of coagulation factors.

Lison et al. (76) conducted a study measuring CCT, pro- and anticoagulants, thromboelastometry and multiplate electrode aggregometry in fifty-one patients undergoing major surgery. Surgeries included vascular surgery, oral or maxillofacial tumor surgery, gynecological tumor surgery and major trauma surgery. On POD1, their study results showed a decrease in platelet count, PT and aPTT within normal range, decrease of AT (but still within normal range). Coagulation factors II, VII, X, XI, XII, and XIII decreased while the acute phase proteins vWF, fibrinogen, and factor VIII increased. Thromboelastometry showed an increase in clot firmness between postoperative day two to six, however it stayed within the normal range. Summarized, results presented by Lison et al. show a change in hemostasis towards hypercoagulability. As they did not analyze the changes in respect to the different surgeries, one cannot say if changes in hemostatic parameters vary among different types of surgery, or if they are equally changed despite the specific procedure. Also, as the study population was small, no general conclusion should be drawn.

4 Conclusion and Discussion

Overall, it has been shown that patients undergoing hepatectomy are most likely to present with an increase in INR/PT after surgery, while measurements of TGA and VET revealed either a balanced hemostasis or a tendency towards transient hypercoagulability.

Levels of most procoagulant factors, with exception of factor VIII, have been shown decreased in most patients undergoing hepatectomy for underlying liver disease (62,64,66,68). In consequence, values for INR/PT have been shown elevated, suggesting a hypocoagulable state. However, also levels of procoagulants protein C and S have been shown decreased. The reason for these measurements is primarily seen in a reduction of hepatocytes, leading to a reduced capacity in the production of both pro- and anticoagulant factors, as well as in a reduced hepatic clearance capacity. This postulate is strengthened by a significant association between major hepatectomy, or respectively lower remnant liver volume percent, and observed coagulopathy (66).

A resulting net balance of hemostatic parameters within the normal range has been shown by TGA and VET in some studies (31,60,64,68). However, a (transient) hypercoagulable state, as identifies by TGA or VET, has been presented by others (61,62,67). This transient hypercoagulability is postulated to be due to an imbalance in coagulation factors, favoring procoagulant ones. It is to be assumed that each coagulation factor shows a different level of decrease after loss in liver function due to individual levels in half-life dependent decay and consumption, as well as time to return to baseline levels (60). E.g., Mallett et al. (64) have shown procoagulant levels returning to normal by POD5, whereas levels of protein C and antithrombin still remained decreased at that point.

Another contributing factor to hemostatic changes has been seen in systemic inflammation accompanying advanced stages of disease and/or surgery, leading to activation of acute phase proteins e.g., fibrinogen, vWF, factor VIII, that promote the TF-dependent activation of coagulation (58) (76). While other procoagulants were mainly seen decreased, factor VIII and vWF were seen increased in two studies (see Table 13), what could be because of their role as acute phase proteins (60). However, fibrinogen was not seen increased but decreased (see

Table 13). Also, the increase in factor VIII could possibly be due to inflammatory processes or due to less inhibition by decrease in proteins C and S.
While intraoperative blood loss and hemodilution have been small due to improved operation techniques and were mainly seen as contributors to decrease in platelet count, they must be kept in mind as extrahepatic mechanisms contributing to hemostatic parameters.

Table 13 summarizes the observed changes in pro- and anticoagulant factors after hepatectomy.

Table 13: Coagulopathy after hepatectomy

	Factor changes promoting bleeding		Factor changes promoting clotting	
Platelets	PC ↓	Decrease measured by: (60) (64) (23) (65) (66) (67) (69). Aktas (46) observed no thrombocytopenia.	vWF↑	Increase in plasma levels and/or functional activity measured by (64) and (23). Marginal change within normal range noted by (68).
	Platelet activation	Not measured	ADAMTS13↓	Decrease in activity measured by (23).
	NO ↑	Not measured		
	Prostacyclin ↑	Not measured		
Coagulation factors	F. II ↓	Decrease measured by (62) and (64). Normal ranges noted by (66) and (68).	Protein C ↓	Decrease measured by (60), (62) and (64).
	F. V ↓	Decrease measured by (64).	Protein S ↓	Decrease measured by (60) and (64).
	F. VII ↓	Decreased measured by (64) (66) and (68).	F. VIII ↑	Increase measured by (62) and (64). No change to baseline noted by (68).
	F.X ↓	Decrease measured by (64). Normal range or no change noted by (66) and (68).		
	F. XI ↓	No change noted by (68).		
	Fibrinogen ↓	Decrease during or shortly after surgery measured by (60), (64), (31) followed by an increase. Normal during surgery and increased on POD1 and 3 as measured by (68).		

Fibrinolysis	TAFI ↓	Decrease measured by (63).	Plasminogen ↓	Decrease measured by (63).
	t-PA ↑	Not measured	PAI ↑	Increase measured by (63).

VET allow a more holistic assessment of hemostasis that is closer to in vivo conditions, by taking both pro- and anticoagulants, fibrinolytic factors, as well as platelets and erythrocytes into account. CCT, especially INR/PT (only assessing procoagulants), have been shown to be no adequate measurement techniques of coagulopathy (64) and therefore should probably mainly be used for surveillance of coumarin therapies. Transfusion of blood products in reply to an elevated INR, when in fact there is no underlying hypocoagulability, could instead promote bleedings by increasing intravascular volume and blood pressure (79).

The presented (transient) hypercoagulable state is potentially effecting vascular health by promoting (micro)thrombosis and thromboembolism. In consequence, damage not only to the vessels (e.g. inflammation, post-thrombotic syndrome) but to all other organ systems can follow (e.g. ischemia).
Also, as described earlier, Prof. A. Haverich postulated the less common hypothesis of vascular inflammation or microthrombi within the vasa vasorum being responsible for atherosclerosis (16). Taking this hypothesis, a hypercoagulable state after liver surgery could promote atherosclerosis and therefore cause major cardio-vascular events.

Limitations

The first limitation is the small number of studies included in Section B. This shortage in studies within recent years has not allowed for a significant conclusion to be drawn. But more importantly, these studies have only performed CCT. None has performed TGA, VET or has measured levels of individual pro- or anticoagulant factors. It would have been of special interest, how levels of protein C and S, factor VIII, AT, vWF, and ADAMTS13 had changed perioperatively to hepatectomy in healthy livers compared to the ones measured in Section A. Therefore, this review unfortunately lacks a comparison of VET and TGA in hepatectomy between diseased and healthy livers. However, looking at cirrhotic vs. non-cirrhotic livers in Section A (the latter being still diseased), it was to note that Jacquenod et al. (66) has seen a significant association of pre-surgery liver

cirrhosis with post-surgery coagulopathy. In contrast, Tanner et al. (67) have not seen a difference between patients with preexisting liver cirrhosis and non-cirrhotic patients regarding a deranged coagulation towards a hypercoagulable state post hepatectomy.
However, it needs to be mentioned, that some studies published in earlier years than 2015 have performed VET in living liver donors. Noting that, it is surprising to see no VET or TGA having been performed in studies of Section B. E.g., Mohammed et al. (2013) (80), Gouvêa et al. (2009) (81), and Cerutti et al. (2004) (82) all have measured elevated CCT in living liver donors, suggesting hypocoagulability, while measurements by VET have been in disagreement. Gouvêa et al. (2009) (81) and Mohammed et al. (80) have shown no significant change in coagulopathy based on ROTEM, while Cerutti et al. 2004 (82) noted hypercoagulability based on TEG parameters. Taking these study results and the earlier postulated underlying pathomechanisms, it is assumable that also hepatectomy performed in healthy living liver donors leads to a state in hemostasis that favors a transient hypercoagulability.

Only Gordon et al. (61) directly compared hemostasis post-hepatectomy in benign and malignant liver lesions and postulated the observed relative hypercoagulable state was not due to malignancy. To investigate this postulate further, more studies with a similar study design, or studies performing VET in healthy liver donors are needed.

A further limitation is the application of thromboprophylaxis. In most studies of Section A, and in 2 out of 3 studies of Section B, patients have received thromboprophylaxis. Most of them have been administered pharmaceutical prophylaxis and some additional mechanical one. In one study of Section A, patients have received mechanical prophylaxis only (Singh et al. (65)). Jacquenod et al. (66) and Aktas et al. (46) have not mentioned, whether patients have received any prophylaxis. The initiation of application and the applied doses have differed between studies, and in some settings it has been upon the treating physician to decide when to start medication (68). Mallett et al. (64) have observed thromboembolism in patients with delayed start of medical thromboprophylaxis. Therefore, when it comes to interpretation of the incidence of thrombosis and

thromboembolism, it needs to be considered that no control group (with no applied thromboprophylaxis) has existed.

While it seems like VTE occurred more frequently than bleedings post-surgery, neither all studies reported about the incidences nor pointed out whether they followed up for these complications at all. Therefore no significant conclusion can be drawn.

Main conclusions

Summarizing, following conclusions can be drawn:

- Hemostatic changes accompanying liver surgery are complex.
- Hepatectomy is often followed by a (transient) hypercoagulable state (61,62,64,66–68), possibly affecting vascular health by promoting thrombosis and thromboembolism leading to vascular occlusion and organ damage.
- Especially, misbalances in the protein C to factor VIII ratio, and vWF to ADAMTS13 ratio have often been seen responsible for hemostatic changes towards hypercoagulability after hepatectomy (23,64)
- The changes in parameters of hemostasis have been shown to be more pronounced after major than minor hepatectomy (66), and to be less pronounced after other abdominal surgeries (23,62,63)
- CCT are not adequate for assessing hemostasis and coagulopathy in liver surgical settings. Thromboprophylaxis should not be withhold from patients based on an elevated INR only (62). VET instead of CCT should be used for decision making regarding blood product transfusion (24,62).
- The possible need for extended thromboprophylaxis in liver surgery should be further investigated (64)

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