Diplomarbeit

IMMOBILIZATION INDUCED COAGULATORY CHANGES: A LITERATURE REVIEW

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Graz, 02.09.2014

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Graz, 02.09.2014 *Adelina Tmava eh*

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ABBREVIATIONS

ADP	Adenosindiphosphat
AP	Alpha antiplasmin
APC	Activated protein C
aPTT	Activated partial thromboplastin time
ATP	Adenosintriphosphat BCP B-cell progenitor
BMI	Body mass index
Ca^{++}	Calcium ions
CBV	Central blood volume
CLP	Common lymphoid progenitor
CMP	Common myeloid progenitor
CO	Cardiac output
Cox 1	Cyclooxygenase-1
DA	Diplomarbeit
DD	D-dimer
DVT	Deep vein thrombosis
EP	Erythroid progenitor
EPO	Erythropoietin
F1+2	Prothrombin Fragment 1+2
FDP	Fibrin split products;
FI	Factor I (Fibrinogen)
FII	Factor II (Prothrombin)
FIII	Factor III (Tissue factor)
FIV	Factor IV (Calcium)
FV	Factor V (Labile factor)
FVII	Factor VII (Proconvertin)
FVIII	Factor VIII (Antihaemophilic factor)
FIX	Factor IX (Christmas factor)
FX	Factor X (Stuart–Prower factor)
FXI	Factor XI (Plasma thromboplastin antecedent)
FXII	Factor XII (Hageman factor)
FXIII	Factor XIII (Fibrin-stabilizing factor)
F-xa	Factor x activation
FVC	Forced vital capacity
FVC1	Forced expiratory volume in 1 sec
G-CSF	Granulocyte-Colony Stimulating Factor
GM-CSF	Granulocyte-macrophage colony-stimulating factor,
GMP	Granulocyte–macrophage progenitor
GPIa/IIa	Glycoprotein Ia/IIa complex (Integrin $\alpha 2\beta 1$)
GPIIb/IIIa	Glycoprotein IIb/IIIa complex (Integrin $\alpha IIb\beta 3$)
HMWK	High molecular weight kininogen (Fitzgerald factor)
HR	Heart rate
HSC	Hematopoietic stem cell
HUT	Head up tilt
IL-x	Interleukine-x
KLF	Krüpel-like transcription factors
LBNP	Lower body negative pressure
MAP	Mean arterial pressure

MBP	Mean blood pressure
mcL	Microliter
M-CSF	Macrophage colony-stimulating factor
MEP	Megakaryocyte erythroid progenitor
MkP	Megakaryocyte progenitor
n	Number of subjects
NE	Noradrenalin
NK	Natural killer cells
NO	Nitrogen monoxide
PAF	Platelet activating factor
PAI	Plasminogen activator inhibitor
PAI-1	Plasminogen Activator Inhibitor 1
PAR1	Protease-activated receptor 1
PAR4	Protease-activated receptor 4
PC	Protein C
PCAT-NR	Protein C activation time-normalized ratio
PDGF	Platelet-derived growth factor
PDGV	Platelet derived Growth Factor
PE	Pulmonary embolism
PF4	Platelet factor 4
PGI_2	Prostacyclin
PK	Pre-kalikrein
PL	Platelet membrane phospholipid
PS	Protein S
PT	Prothrombin time
PT	Prothrombin time and
RBC	Red blood cells (Erythrocytes)
ROTEM®	Rotational thromboelastometry
SCF	Stem cell factor
SV	Stroke volume
TAFI	Thrombin Activatable Fibrinolytic Inhibitor
TAT	thrombin-antithrombin III complex
TF	Tissue Factor
TFPI	Tissue factor pathway inhibitor
TGF β	Transforming Growth Factor Beta
TM	Thrombomodulin
TNK	T-cell natural killer cell progenitor
TPA	Tissue plasminogen activator
t-PA	Tissue plasminogen activator
XL-Fibrin	Cross-linked fibrin
TPO	Thrombopoetin
TSP	Thrombospondin
TSP-1	Thrombospondin 1
TxA2	Thromboxane A2
UK	Urokinase
u-PA	Urokinase Plasminogen Activator
VO2 max	Maximal oxygen consumption
VTE	Venous thromboembolism
vWF	Von-Willebrand Factor
$\alpha_5\beta_1$	Alpha -5 beta-1 Integrin

βTG	Beta-Thromboglobulin
5-HT	5-hydroxytryptamine
6^{o} HDT	6 degree head-down tilt
↑	Increase
↓	Decrease

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ABSTRACT

Bed rest immobilization has been suggested to affect coagulation. However, up to today there is no consensus on the reported data: some studies suggest that there is a tendency to hyper clot while others have not reported such effects of immobilization.

This diploma thesis explores the physiological basis of coagulation, and then discusses current literature that shows how immobilization effects coagulation. The aim is to search the existing literature and provide current state of the art knowledge with regards to how coagulation is regulated, paying particular attention to anti- as well as pro-thrombotic mechanisms. Is it possible that the increased tendency to clot is complemented by increased anti-thrombotic activity, leading to no net effects in coagulation during immobilization? This diploma thesis will search for the latest articles that show what happens during bed rest immobilization with particular emphasis on coagulation. The effects of different postural changes as well as stress hormones and sitting immobilization on coagulation will be explored. The relationship to differences in methodologies, subject/ patient selection, types of interventions as well as differences in protocols are going to be discussed.

To obtain the relevant literature related to my topic I used PubMed as primary database. Explored were articles written in English or German, publicized from 1957 till 2014. Abstracts plus full texts were included as primary literature. Furthermore, Web of Science database as well as the list of References were also used to find articles related to the area of search. Books particularly physiology and pathophysiology books were used as secondary literature. Refworks was used to save and organize the references.

The knowledge gained from this work would be particularly relevant to those future medical practitioners who have an interest in working in the area of falls, orthopedics or thrombosis research.

ZUSAMMENFASSUNG

Es wird angenommen, dass die Bettruhe und Immobilisation die Blutgerinnung beeinflussen. Es gibt derzeit aufgrund der Datenlage jedoch noch keinen Konsens über diesen Zusammenhang: einige Studien deuten auf eine Tendenz zur Hyperkoagulation hin, während andere keine Auswirkungen der Immobilisation auf die Blutgerinnung feststellen konnten.

Diese Diplomarbeit erforscht die physiologischen Grundlagen der Blutgerinnung und diskutiert darüber hinaus die aktuelle Literatur, die darstellt, wie Immobilisation die Hämostase beeinflusst. Ziel dieser Arbeit ist es, die bestehende Literatur zu erforschen und den aktuellen Wissensstand in Bezug auf die Regulierung der Blutgerinnung zu setzen. Besondere Aufmerksamkeit wird auf anti- sowie pro-thrombotischen Mechanismen gelegt. Diese Diplomarbeit basiert auf den neuesten Publikationen über die Bettruhe und Immobilisation mit besonderem Schwerpunkt auf den Prozess der Blutgerinnung. Ebenso werden die Auswirkungen verschiedener Faktoren, wie Haltungsänderungen, Einfluss der Stresshormone und die Sitz-Immobilisierung auf die Hämostase erforscht. Unterschiede in der Methodik, Subjekt / Auswahl der Patienten, die Art der Interventionen, sowie die Unterschiede in den Protokollen werden diskutiert.

Um die relevante Literatur für meine Diplomarbeit zu finden, habe ich PubMed als primäre Datenbank verwendet. Verwendung fanden englisch- und deutschsprachige Publikationen, die zwischen 1957 bis 2014 veröffentlicht wurden. Abstracts und Volltexte wurden als Primärliteratur verwendet. Außerdem wurde auch die Datenbank Web of Science sowie die Liste der Referenzen verwendet, um relevante Artikel für das Thema zu finden. Es wurden auch Bücher, besonders Physiologie und Pathophysiologie Bücher als Sekundärliteratur verwendet. Gespeichert und organisiert wurden die Referenzen mit dem Programm Refworks.

Die Erkenntnisse aus dieser Arbeit können besonders relevant für zukünftige Ärzte sein, die sich für die Forschung in den Bereichen der Unfallmedizin, der Orthopädie oder der Thrombose interessieren.

I. INTRODUCTION

1. HEMOSTASIS

The ability of the blood to prevent its loss when a vessel is severed or ruptured is called coagulation. On the other hand, when there is no need for it, the opposite effect dominate on a blood stream. Coagulation and Anticoagulation are the functions of a defend blood reaction known as Hemostasis (1). There are a lot of substances incorporated on the hemostasis, some of them promote coagulation and the others inhibit it. The balance between pro- and anticoagulants is essential to maintain the hemostasis into physiologic frames. To explore the whole process of physiologic hemostasis, the main domains involved on it are going to be discussed.

1.1. Vascular wall

It is an active component of the hemostasis which helps to maintain anticoagulation state on the blood as well as to initiate coagulation when necessary. It consists of three layers: adventitia, media and intima. Intima is composed from squamosepithelcells called endothelcells. They play a central role on the whole hemostasis. An intact endothelium is a premise of anticoagulation effects of the vascular wall. It serves as a border between blood components and sub - endothelial coagulation promoters (Tissue factor TF, collagen). The production and secretion of anticoagulants like Thrombomodulin and Heparin sulfate as well as secretion of platelet inhibitors like

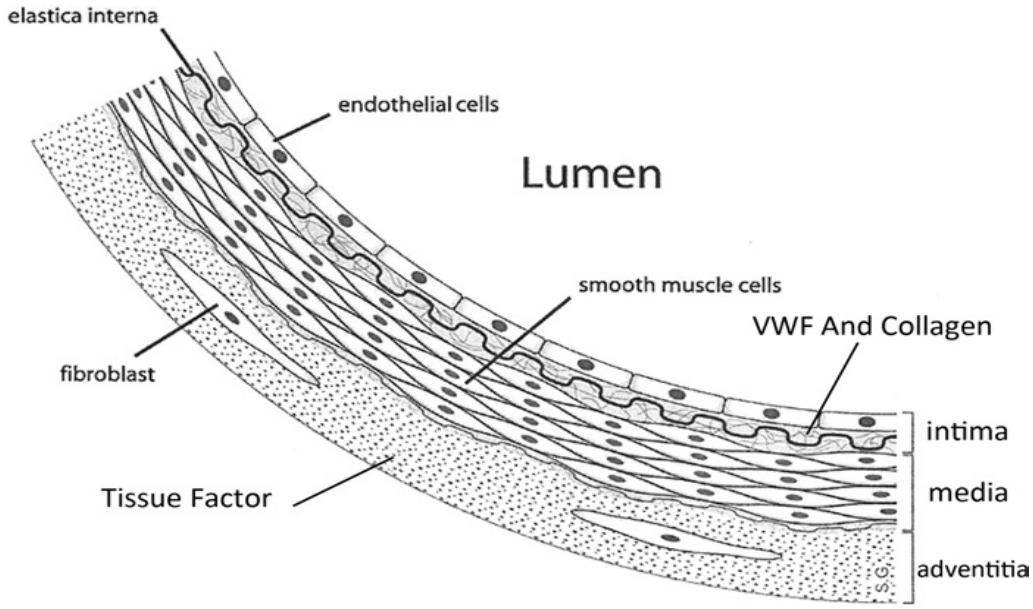

Figure 1: Layers of the vascular wall (10)

Prostacyclin (PGI_2) and nitrogen monoxide (NO) are some other effects of this layer. Endothelial cells impact also fibrinolysis by secretion of Tissue type Plasminogen Activator (t-PA) and Urokinase type Plasminogen Activator (u-PA) as well as secretion of Plasminogen Activator Inhibitor (PAI-1).

As already mentioned, vascular wall contribute on blood coagulation. The first reaction that occurs after a blood vessel has been cut or ruptured is vasoconstriction (2). The smooth muscle of the damaged vessel contract so the flow of blood will be reduced thereby the blood loss too. This reaction results from local myogenic spasm, local autacoid factors from the traumatized tissue and blood platelets as well as nervous reflexes initiated by pain nerve impulses or other sensory impulses. Die irritated nerves have a positive effect on adrenalin secretion from the adrenal medulla thus inducing also a vasoconstriction (3).Vasoconstriction can last min to hours, at the same time coagulation happens (2). When the endothelial layer lose its integrity, subendothelial collagen will be exposed to the blood stream thus initiating platelet adhesion and aggregation throw von-Willebrand Factor. Tissue Factor is another component of the vascular wall present on fibrocytes, macrophages and myocytes of the tunica media and adventitia. Its exposure to the blood trigger the coagulation cascade (1).

Table 1: Components of the vascular wall, translated from Barthels, 2013 (1)

Substance	**Function**
Collagen	Adhesion and aggregation of the platelets
Prostaglandin (PG) End product: Prostacyclin (PGI_2)	Inhibition of the platelet aggregation
Heparane (Heparansulfat)	Anticoagulation on endothelium
Thrombomodulin	Cofactor from Thrombin to activate the Protein C
Von-Willebrand-Factor (vWF)	Connector between sub endothelium and platelet Defend the Factor VIII from early degradation
Tissue Factor (TF)	Activation of the coagulation throw Complex formation with Factor VIII
Tissue type Plasminogen Activator (t-PA) and Urokinase type Plasminogen Activator (u-PA)	Activation of fibrinolysis
Plasminogen Activator Inhibitor PAI-1	Fibrinolysis inhibitor

1.2. Platelets

They are cell fragments of the megakaryocytes formed on the bone marrow. Megakaryocytes derived from the hematopoietic stem cell. Thrombopoetin, a protein hormone produced and secreted from the liver, stimulate parallel the growth as well as the differentiation of the megakaryocytes (4) . During maturation of these cells, they replicate the number of nuclei together with cytoplasm enlargement but without cellular division (2). In the bone marrow or after entering the blood, megakaryocytes break up into platelets (see Figure 3).

Under physiologic condition, the number of platelets in the blood is 150000- 350000/mcl. 75% of these new platelets remain to the blood; the others will be pooled in spleen (4). The nucleus reminded at the end, will be transported to the lung and destroyed there throw macrophages. A half-life of the platelet is 8-12 days than it is eliminated from the circulation throw the tissue macrophage system (dominantly in the spleen) (2).

The form of the platelets depend on their activity. When they are not active, they have a discoid form. After activation, their aktin-myosin cytoskeleton contract and the platelets become an irregular form with pseudopodia, consequently increasing their surface. During this transformation, phospholipids of their membrane change their configuration, so the acidic phospholipids switch to the outer layer of platelet membrane (Flip-flop mechanisms) (5). Acidic phospholipids serve as receptors for the coagulation factors thus promoting the coagulation.

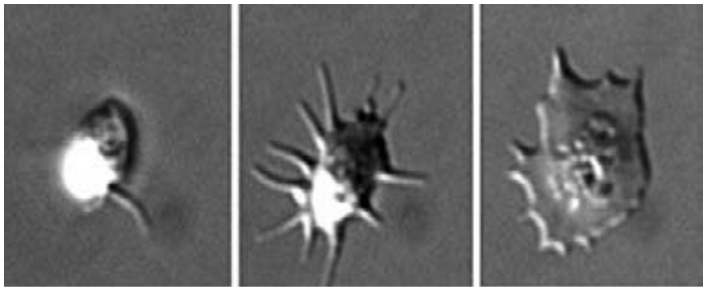

Figure 2: Platelet morphologic change viewed with electron microscope (4)

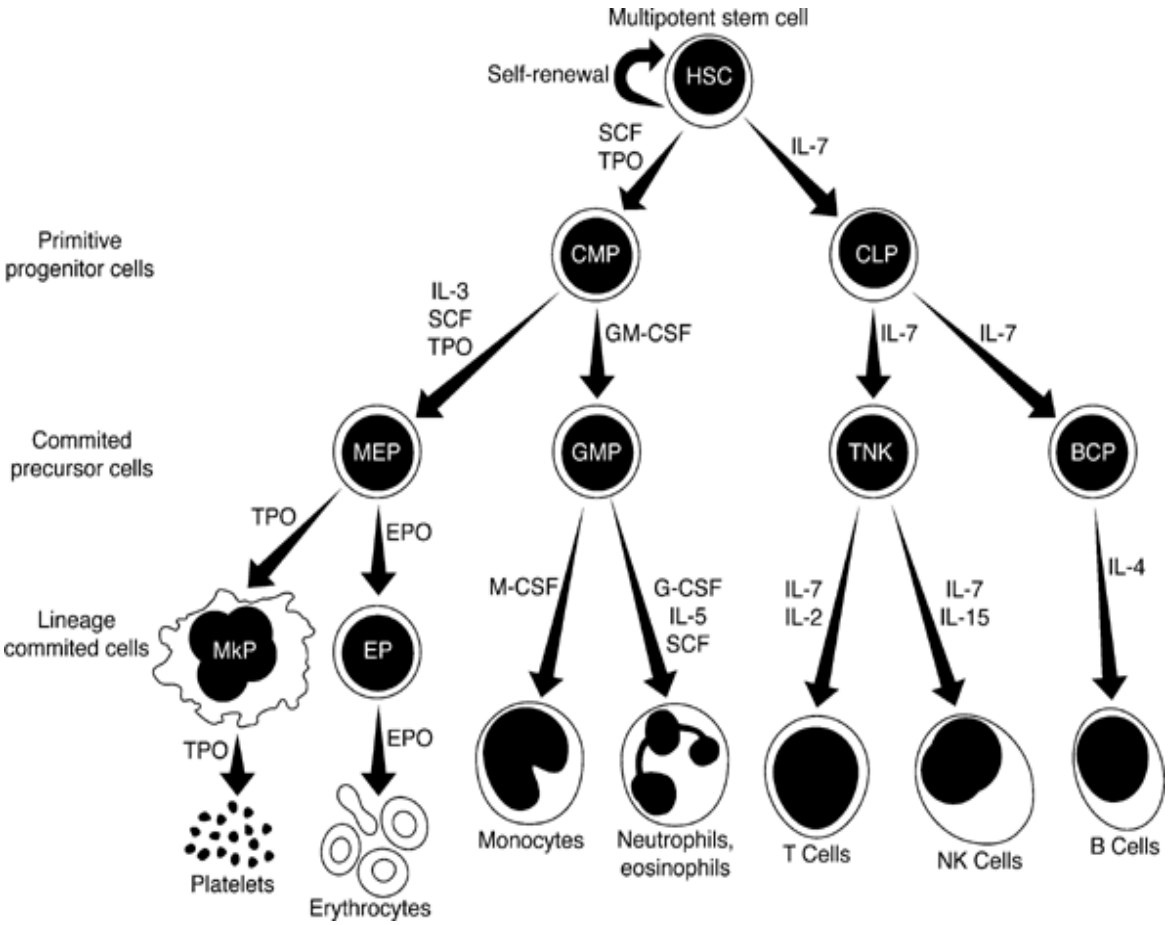

Figure 3: Hematopoiesis (6); **Legend:** BCP: B-cell progenitor; CLP: common lymphoid progenitor; CMP: common myeloid progenitor; EP: erythroid progenitor; HSC: hematopoietic stem cell; GMP: granulocyte–macrophage progenitor; MEP: megakaryocyte erythroid progenitor; MkP: megakaryocyte progenitor; TNK: T-cell natural killer cell progenitor.

Even that the thrombocytes do not have nuclei, they can produce proteins throw their mRNA cytoplasmic and mitochondria. On their cytoplasm, platelets have also granules filled with agents that impacts whole hemostasis. Types of the granules and their content are figured on the Table 2.

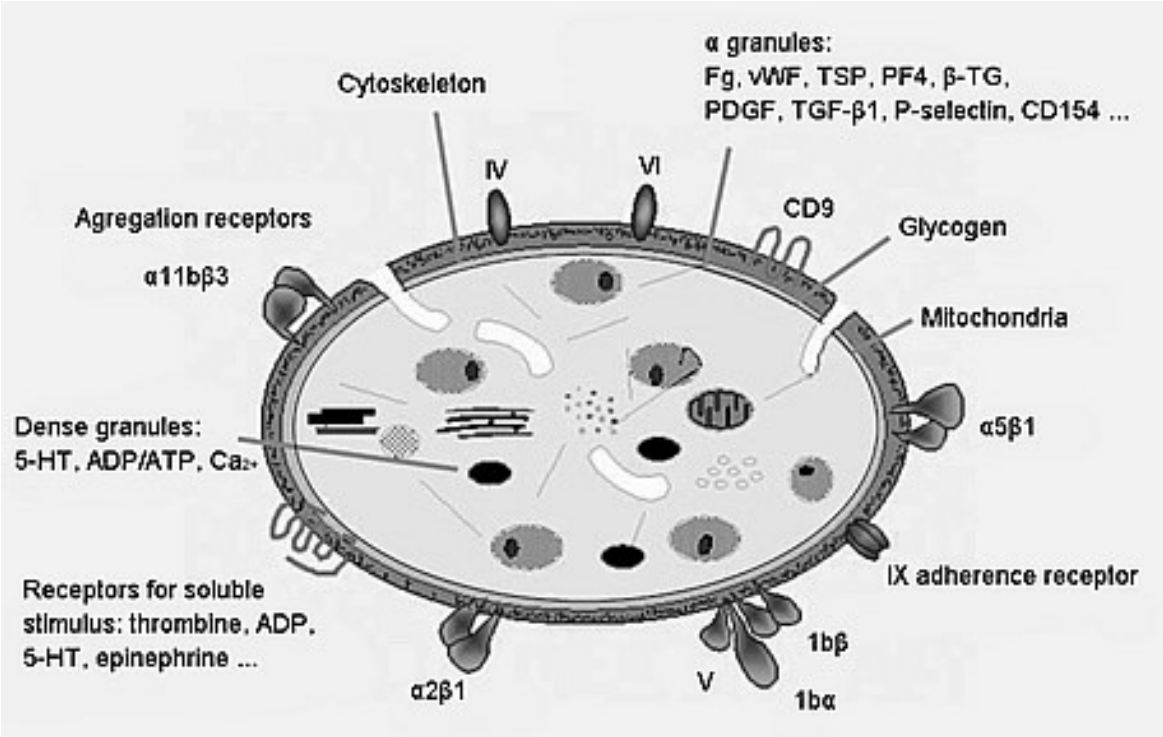

Figure 4: Platelet structure (7)

Table 2: Granules of a platelet, translated from Ganser, 2013 (4)

α-granules	
Adhesion proteins	Fibrinogen, von Willebrand factor, Thrombospondin-1 (TSP-1), Fibronektin, Vitronektin
Procoagulation Factors	Factor- XII, Factor V, Factor XI, Matrixmetalloproteinase, Kininogen, Calcium
Anticoagulation Factors	TFPI, Protein C, Protein S, α_2Macroglobulin, α_1Antitripsin, Matrixmetalloproteinase, Platelet factor 4 (PF4) as Cofactor of Protein C system
Components of Fibrinolytic system	Plasminogen, Plasmin-Inhibitor, Plasminogen Activator Inhibitor
Chemokine, Immune mediators	Platelet factor 4 (PF4), β-Thromboglobulin, Interleukin- 1β, complement factors, Platelet derived Growth Factor (PDGV), Transforming Growth Factor β (TGF β), Platelet activating Factor(PAF)
Membrane proteins	P-Selectin, α2β1-Integrin, $\alpha_{2b}\beta_3$-Integrin, CD36
Dense Granules or Phospholipid-layer δ	

Nucleotides that influence the platelet activation	ADP: Platelet activation and recruitment ATP: (Precursor of ADP): Influence the cytoskeleton
Other mediators	Serotonin: Influence the vascular tonus ATP: Activate leukocytes
Cations	Calcium, Magnesium
Lysosomes	
Proteases	among others: Elastase, Collagenase, Carboxypeptidase

1.2.1. Platelet plug formation

This mechanism is important for closing minute ruptures in very small blood vessels occurring many thousands of times daily (2). The classical model of platelet plug formation is based on two phases occurring successively: platelet adhesion and platelet aggregation. This process results in thrombus formation, a structure that became stabile throw fibrin fibers. The substances that promote thrombus formation are: collagen, thrombin, thromboxane A2, ADP, fibrinogen bound to $\alpha_{2b}\beta_3$-Integrin, vWF bound to $\alpha_{2b}\beta_3$-Integrin, and epinephrine. Prostacyclin (PGI_2) counteract this reaction (4).

Platelet adhesion – when the endothelium of a vessel is damaged, the sub endothelial collagen is exposed to the blood stream. This exposure attracts platelet to bind to collagen as well as sub-endothelial receptor- vWF. Binding of collagen to the platelet receptors α2β1-Integrin activate the platelets (4). They change the form, from discoid to irregular, membrane phospholipid configuration change, the production of Thromboxane A2 is induced as well as the platelet aggregation is initiated.

Platelet aggregation- after the activation, platelet receptors $\alpha_{2b}\beta_3$-Integrin linked throw fibrinogen bridges as well as throw vWF, holding together the platelets with each other and with sub-endothelium.(4) The whole process together lead so within 1 min to platelet plug formation (2).

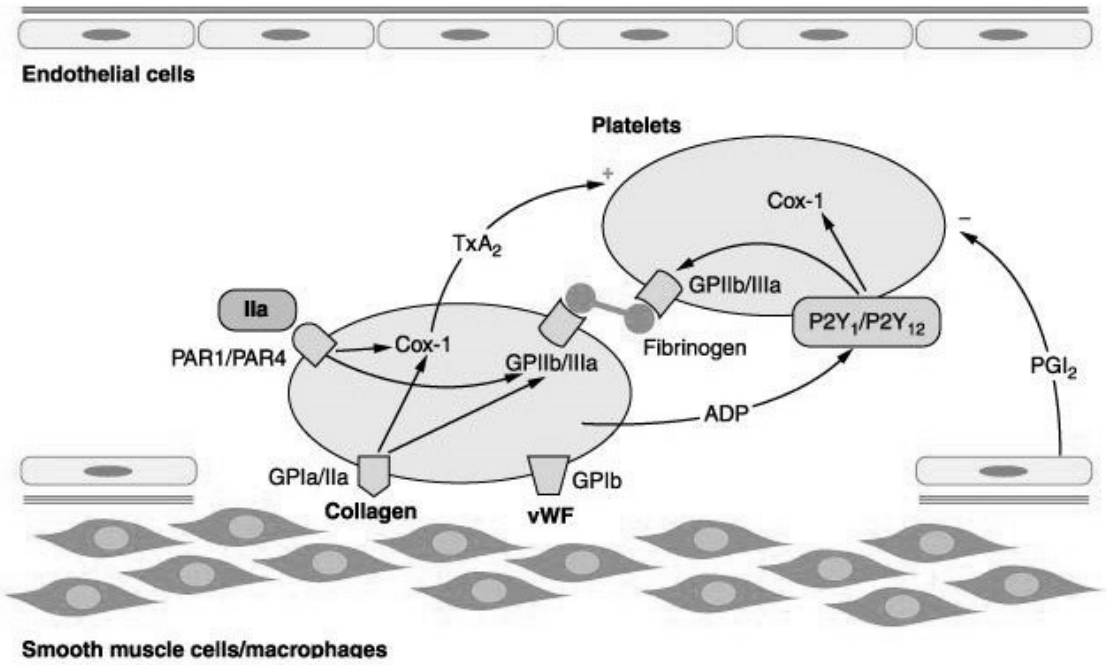

Figure 5: Platelet adhesion and aggregation (8)

Based on the studies done at the last time, using in vivo models, an overlap of platelet plug formation and blood coagulation has been evidenced(9). This new topic, that dissents the classic model of thrombus formation, explains two possible pathways of platelet activation (10). The first mechanisms is considered to be the exposure of sub endothelial collagen as trigger for the platelet activation, just like the classic model also describes. Another mechanisms that potentially activate platelets is shown to be the exposure of tissue factor (TF), a membrane protein found especially on tunica media and adventitia of the vascular wall, which also initiate thrombin formation. These two independent arts of platelet activation and aggregation seems to depend on magnitude of the injury (11). When the endothelial break occurs, than the sub-endothelial collagen way appears to be the first line of reaction. When the injury get to the deeper layers of vascular wall than the platelet activation as well as thrombin generation is going to be induced throw the Tissue Factor (TF) pathway.

Beside the evolution of platelet activation and aggregation mechanisms, also the importance of vWF and Fibrinogen as adhesive molecules is being explored. The experiments done on the mice showed the ability of the platelets to create thrombi even in the absence of vWF and Fibrinogen (12). Significantly was the fact that the thrombi created without the presence of vWF and Fibrinogen were not stable and had a tendency to

embolism. These results submit the possibility of existing von new adhesive molecules not known till yet, that take part on the interaction between platelets and endothelium (12).

1.3. Blood coagulation models

The platelet plug is not stable and can be washed away. To prevent the re-bleeding and to strength this plug, a cascade complex mechanisms occurs, which at the end will result in the formation of a stable mesh compound mainly from the fibrin strands(2). An interaction of plasma proteins, different cells as well as mediators are necessary to result on blood coagulation. During the history of coagulation literature, many coagulation models came out and tried to explain exactly what happens throw this process. Because of the process complexity, these models evolve even yet, making so the coagulation field still mysterious and attractive for research.

1.3.1. Cascade Model

For a long period of time, the waterfall/cascade model is used to explain the coagulation. It is based on stepwise reaction sequences of inactive serine proteases. During this process, an inactive serine protease (zymogen) with its cofactor act together to become an active complex which catalyze the next reaction (13). This model include two pathways (intrinsic and extrinsic) which are at the beginning independent to each other , than at one point of the cascade they come together and continue as a common pathway. This common pathway results to thrombin formation, a multifunctional substance that catalyze the reaction of converting fibrinogen to fibrin.

The intrinsic pathway (contact pathway) has a central role into the cascade model. How also the name of it suggest, this pathway is called intrinsic, because the constituents needed for it were all present on blood. FXII and pre-kalikrein (PK) together with the cofactor high molecular weight kininogen (HMWK) compound this pathway (10). The first step is thought to be activation (a) of FXII. Which substance is responsible for this activation in vivo, is still not known. The studies done in vitro, showed that the FXII activate itself when it comes in contact with artificial surfaces like plastic, while in vivo the negatively charged substances like collagen or polyphosphate (PolyP) has shown to trigger the activation (10). PolyP, a newly discovered substance, secreted from dense granules of platelets, seems to promote the hemostasis, thrombosis as well as inflammation process (14).

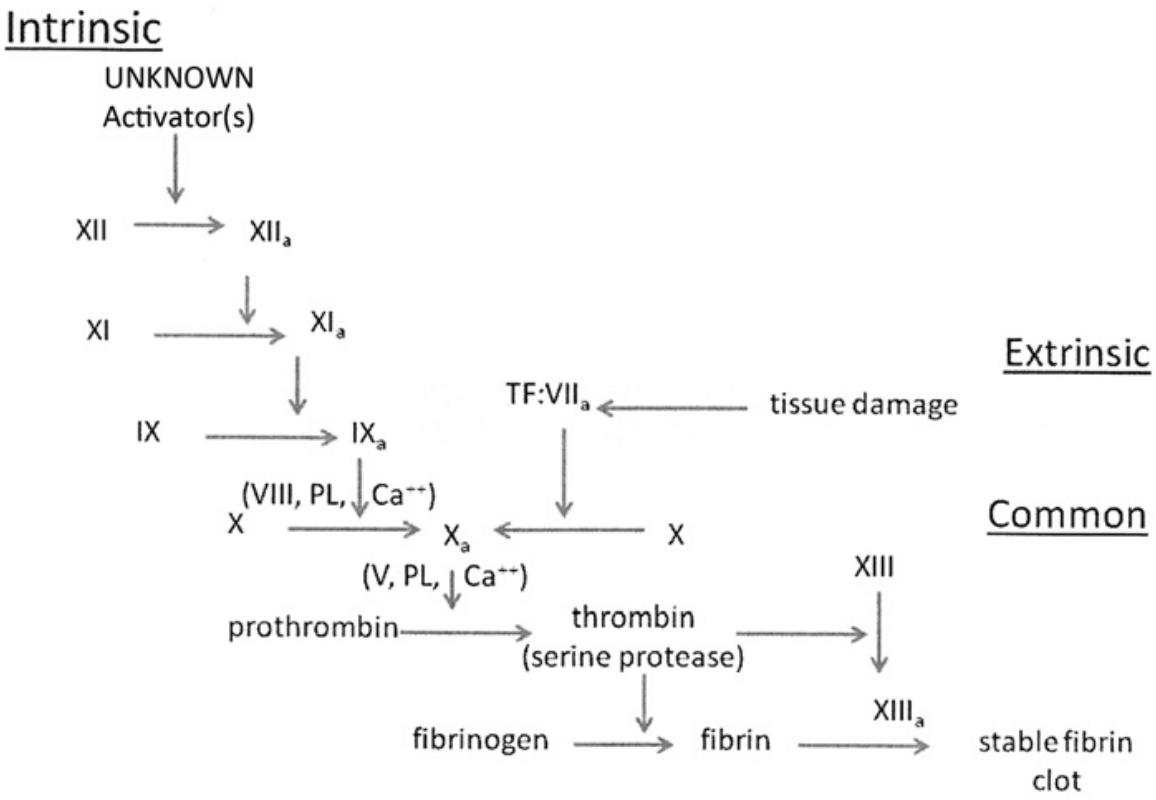

Figure 6: Cascade model of clot formation (10); **Legend**: XII: Hageman Factor; XI: Plasma thromboplastin; IX: Christmas factor; VII: Stable Factor, XII: Fibrin stabilizing factor; PL: Platelet membrane phospholipid; Ca^{++}: calcium ions; TF: Tissue Factor

The activated Factor XII affect not only coagulation, but also the complement system. After being activated, Factor XII catalyze the activation of Factor XI in the presence of HMWK and PK. These first two reactions mentioned above, do not need calcium to promote or accelerate their reactions. Otherwise the reactions that follow, require calcium to take place. Factor XIa induce the activation of Factor IX. Factor IXa in the presence of calcium, phospholipids and Factor VIII, catalyze conversion of Factor X to Factor Xa. Generation of Factor Xa is the point where the intrinsic and extrinsic pathway meet together.

The extrinsic pathway was thought not to be the primary pathway of coagulation, and its importance on this process was underestimated. The trigger of this pathway is damage of vascular wall, thus exposing the tissue factor to blood. Consequently, Factor VII comes in contact with TF and together create an active complex. This complex catalyze the

activation of Factor X. Factor Xa is essential for thrombin formation which catalyze the conversion of fibrinogen to fibrin.

The cascade model reflects the coagulation in vitro. Plasma- based tests used routinely for the coagulation deficiencies diagnostic, are created based on it (10). This model describes the coagulation process that occurs in plasma without taking consideration of endothelial interactions. Based on last studies, it came out that the intrinsic pathway of this model, does not play an essential role for hemostasis in vivo, but it can be important for the pathophysiology of thrombosis (10). Lack of cellular elements in the cascade model set the stage for the new coagulation model discussed below.

1.3.2. Cell- based Model

Despite the fact that cascade model was very helpful in exploring the coagulation, it was not able to answer the questions like: why people deficient on FXII or PK do not have coagulation problems if the intrinsic pathway a central position hat, or when two pathways independent von each other exist, than why bleed people with hemophilia?! The cell based Model presented at first from Mann (15), aimed to give the answers unknown till that time. This model shows the central position of the cell components on the hemostasis in vivo. Phospholipid membrane surface (phosphatidylserine) mainly of the activated platelets is thought to have pro coagulant effects. Also the other cells like endothelial cells, monocytes, smooth muscle cells, seems to have pro coagulant membrane surfaces. The interactions between classically intrinsic and extrinsic pathways is shown to be present, but the presence of the contact factors for coagulation, has been proven to be not necessary (10).

The cell based model is conceived in an initial and a propagation phase.

Initial phase- a break of the endothelium barrier enable the smooth muscle cells, adventitial cells and keratinocytes to express to the blood their membrane protein called tissue factor (TF). The contact of TF with blood makes possible the coagulation in vivo. Although the most of the TF does not normally contact to blood, some studies evidence the presence of its small amount also in plasma (10). What is the role of this plasma TF, is still unknown. Part of the initial phase is also Factor VII, the only Factor present in blood in both active and inactive form even as inactive is only 1% present(16). It is still unknown which is the most significant activator of FVII, but Factor IXa, Factor Xa, Factor XIIa, plasmin, thrombin as well as auto activation can also activate FVII. The contact of TF with

FVIIa result in a complex formation known as TF-FVIIa. This complex consider to be the only activator of the coagulation in vivo under physiologic conditions (10). After activation, the complex TF- FVIIa activates FIX as well as FX. The Factor Xa resulted on this phase is not enough sufficient to maintain hemostasis but it triggers the formation of a trace amount of thrombin (17). This limited action of FXa is closely linked with the powerful inhibition activity of Tissue Factor Protein Inhibitor (TFPI) on TF- FVIIa – FXa complex, as well as inhibition of thrombin and FXa throw Antithrombin (AT)(18). Despite the amount of thrombin generated in this phase is small, it triggers the activation of platelets and their flip-flop mechanisms resulting in generation of a platelet procoagulant phospholipid surface. It also cleaves FXI and FV on the platelet membrane to their active form as well as release FVIII from vWF resulting in FVIII activation. This prothrombic period called amplification set the stage of the next step of coagulation.

Propagation phase- the propagation phase demonstrates the generation of much more thrombin on the surface of platelets. This amount is enough to catalyze the reaction of converting fibrinogen to fibrin. The outcome of activation phase were the activation of FX, FIX as well as cofactors FV and FVIII. Factor IXa react together with FVIIIa and bind to platelet membrane throw calcium ions. This complex is called tenase complex. It is very powerful in activation of Factor X (10). Factor Xa in presence of its accelerator Factor Va and calcium ions compose prothrombinase complex. The formation of prothrombinase complex catalyzes the massive production of thrombin from prothrombin, leading so to formation of fibrin clot.

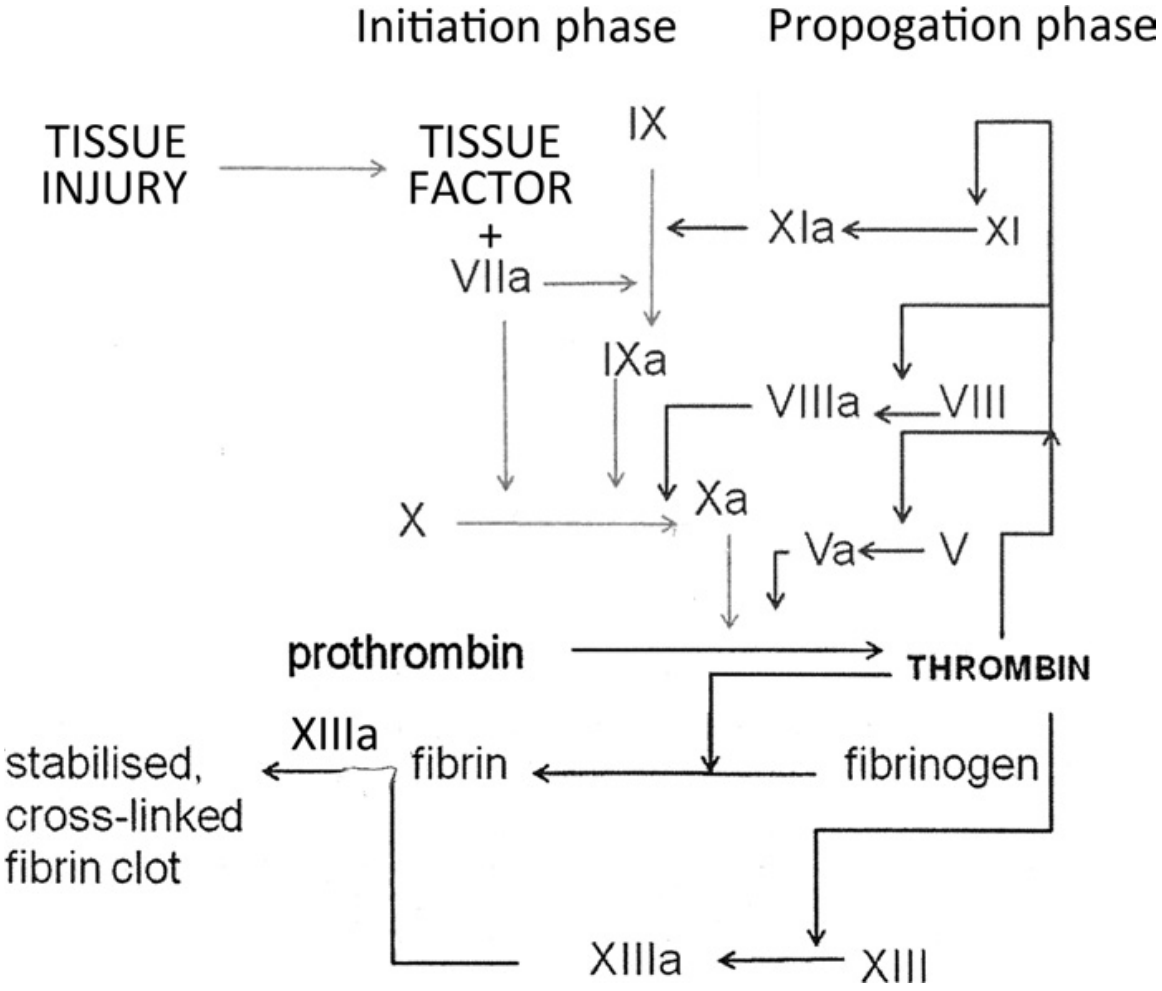

Figure 7: Cell-based Model of coagulation (10)

1.4. Formation of fibrin

Generation of a stable fibrin clot is the next step of coagulation that occurs after formation of thrombin. The substrate of this process is fibrinogen, a polypeptide dimer synthesized on the liver. It is water-soluble therefore it circulates free on blood. Furthermore it is also found extravascular and extracellular thus establishing its function on wound healing as well as inflammation (19). Fibrinogen is an adhesive macromolecule, particular to injured endothelium and platelets membrane. Its influence on the blood viscosity is also known. To convert fibrinogen into fibrin, thrombin cleaves its peptides fibrinopeptid A and B, thus resulting in monomer fibrin formation (19). This form of fibrin is still water-soluble and a mesh has to be formed. Fibrin connect its chains to each other composing so a mesh. Factor XIIIa already activated from thrombin, cross-linked the fibrin fibres and binds the inhibitors of fibrinolysis thus resulting at the end to generation of a stable fibrin clot.

1.5. Physiologic (natural) inhibitors of coagulation

As already mentioned above, to maintain the hemostasis und physiologic frames, the coagulation process is counteracting throw the inhibitors. The main natural inhibitors of clotting are *Serine-Protease Inhibitors (Serpins)*, which irreversibly inhibit the pro-coagulation enzymes by binding to their active centrums (20). The complex Serpine-Coagulation Enzyme bind to a plasma protein called Vitronectin, and in this form will be eliminated either on the liver or on the vascular wall. The functions of serpins are not limited only on coagulation. They also inhibit fibrinolysis (PAI-1 and α2-antiplasmin) as well as Inflammation (α1-Antitrypsin) and Complement (C1-Inhibitor)(20). The main members of this group counteracting pro-coagulatory state are *Antithrombin, Protein C and Proteins S.* Antithrombin mainly inhibits the free form Thrombin and Factor Xa but also affects the Factors IXa, XIa, XIIa, Kalikrein and Plasmin. Based on the fact that antithrombin is one "Progressive Inhibitor", it needs accelerators to express optimal effects. Its accelerators are either heparansulfate of endothelium or heparin (20).
Protein C with its accelerator Protein S inhibit Factor VIIIa and Va throw limited proteolysis. The activation of Protein C makes possible formation of Thrombin-Thrombomodulin complex as well as Endothel-Protein C- Receptor. Protein S enhance the activity of Protein C active form.

Another coagulation inhibitor, not in the group of serpins is *Tissue Factor Pathway Inhibitor* (TPFI). TPFI inhibits reversible Factor VIIa, TF/Factor VIIa complex and Factor Xa thus impacting the initial phase of coagulation described previously. It is secreted mainly from endothelium and circulates on blood in two forms: bound on Liproteins (90%) and free form (10 %)(20). *Heparin Cofactor II, Protein-C-Inhibitor, Protein-Z-Inhibitor, α_1-Proteinase-Inhibitor, C1-Esterase-Inhibitor, α2-Macroglobulin* are also inhibitory proteins that contribute to maintain the hemostasis in balance.

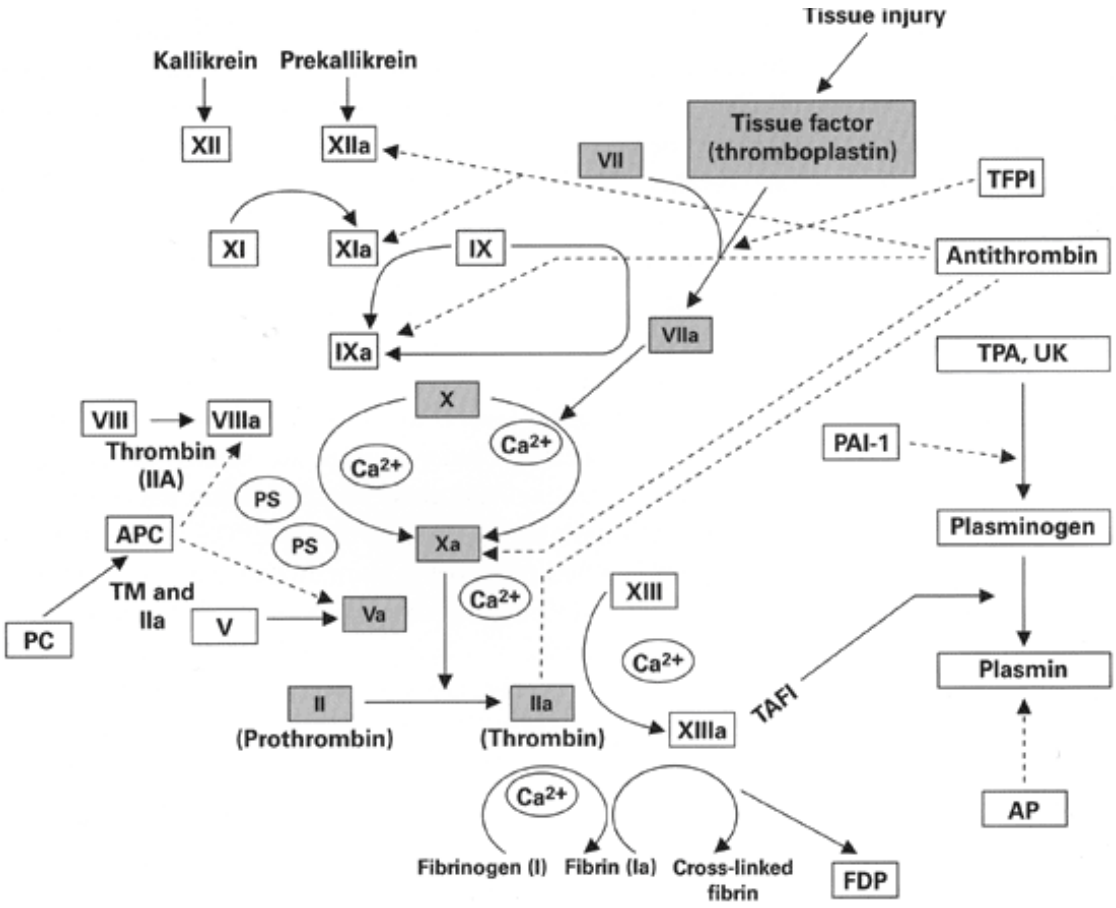

Figure 8: Natural inhibitors of coagulation illustrated on tissue factor dependent pathway (21). **Legend**: Dashed lines (---) represent site of major inhibitors. AP: α-antiplasmin; APC: activated protein C; FDP, fibrin split products; PAI: plasminogen activator inhibitor; PC: protein C; PS: protein S; TAFI: thrombin-activatable fibrinolysis inhibitor; TFPI: tissue factor pathway inhibitor; TM: thrombomodulin; TPA: tissue plasminogen activator; UK: urokinase

Natural inhibitors are not the only group that inhibits coagulation factors. They should not be confused with circulating anticoagulants (acquired inhibitors of clotting), which are antibodies against clotting factors (22). They can be specifically directed against an isolated coagulation Factor like FVIII and IX or against multiple proteins presented from antiphospholipid antibodies (22).

1.6. Fibrinolysis

This process enable dissolution of fibrin clot and recanalization of blood vessel. It occurs throw enzymatic activity of plasmin by acting on fibrin cross-linked fibers as well as fibrinogen. Similar to coagulation under physiological conditions, also the fibrinolysis occurs local on the place where there is need for it. First step is conversion of plasminogen to plasmin. Catalyser of this reaction are: tissue type Plasminogen activator (t-PA) synthesized from Endothelium and Urokinase type Plasminogen activator (u-PA) synthesized from epithelial cells, fibroblast like cells, monocytes and endothelium (10). Tissue type Plasminogen activator (t-PA) is main activator on the blood. To express its optimal activity, it requires except plasminogen also fibrin. Fibrin enhance the activity of t-PA and impact the whole reaction by acting as accelerator of plasminogen activation as well as substrate of plasmin already generated.
Urokinase type Plasminogen activator (u-PA) is main extravascular activator. Its function appears to be on peri-cellular Proteolysis by tumours, function of macrophages etc. Dissimilar to t-PA, u-PA do not require fibrin to activate plasminogen (10).

Enzymatic effect of plasmin on fibrinogen and cross-linked fibrin, results in different degradation products. Fragment D and E are end product of fibrinogen degradation, while the presence of D-dimer fragment is an evidence of cross-linked fibrin degradation (19). Plasmin affects not only fibrinolysis but also the coagulation process by inhibiting prothrombin, Factor XII, Factor XI, Factor IX and Factor VIII.

Inhibitors prevent the unnecessary fibrinolysis and control the overshooting of dissolution. *Plasminogen activator Inhibitor 1 (PAI-1)* secreted from endothelium inhibits direct t-PA as well as u-PA on the blood and takes the place as the main fibrinolysis inhibitor on the blood (19). To stop the binding of plasminogen and t-PA to fibrin, *thrombin activable fibrinolysis inhibitor (TAFI)* after being activated from thrombin-thrombomodulin complex and high thrombin concentration, cleaves fibrin C- terminal side. Another inhibitor is *Plasmin inhibitor (α_2 antiplasmin)*, which inhibit the plasmin by binding to fibrin throw Factor XIIIa during fibrin clot stabilization and also inhibit direct the free plasmin eventually found on blood stream. Inhibition effects of *PAI-II* and *Lipoprotein (a)* have been evidence too (19).

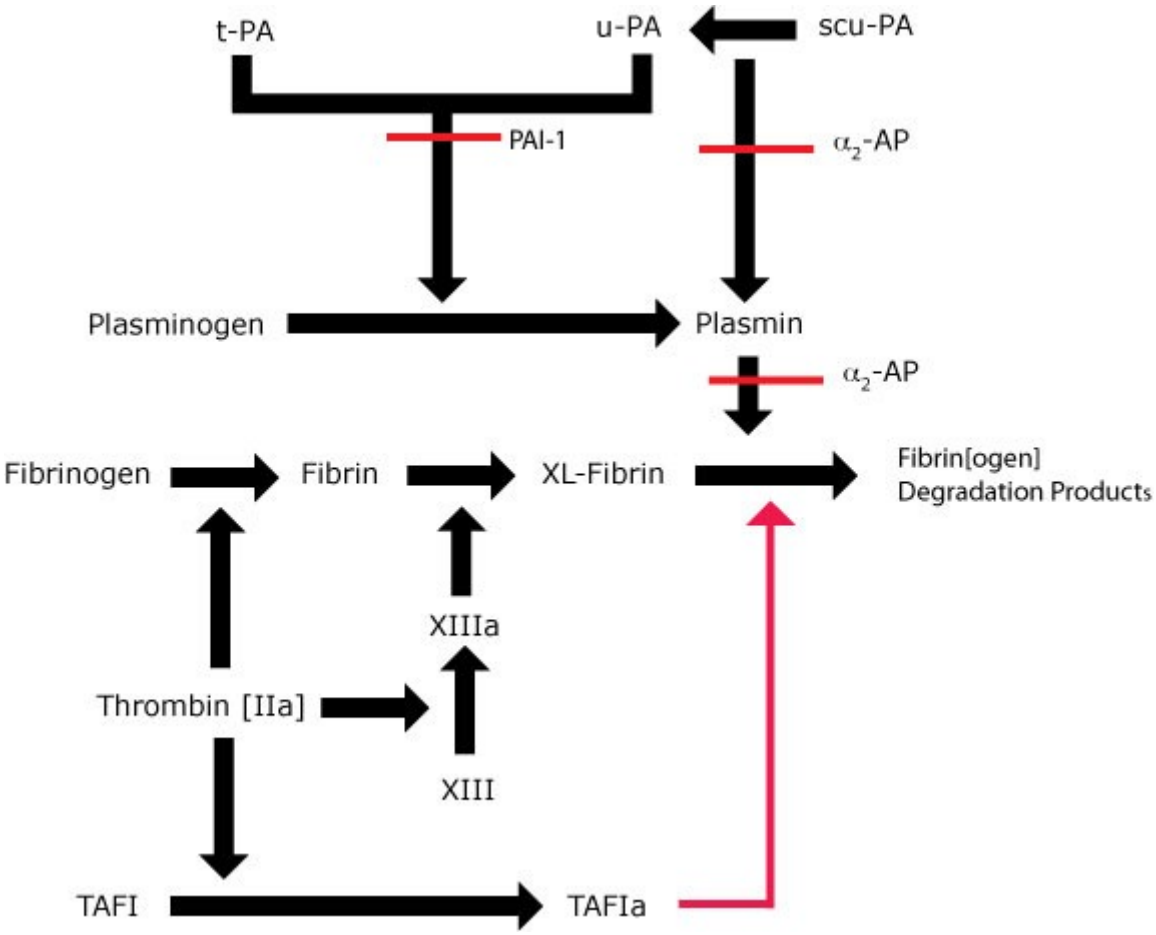

Figure 9: Fibrinolysis (23); **Legend:** t-PA: tissue plasminogen activator; u-PA: urinary plasminogen activator; XL-Fibrin: cross-linked fibrin; TAFI: Thrombin Activatable Fibrinolytic Inhibitor.

The studies done on the mice from Lam et al (24), suggests also another mechanism of fibrinolysis different from plasmin-induced pathway. They found fibrin clots outside of capillary of mice central nervous system, thus suggesting that the endothelium create vesicles around thrombus and then release it outside of the vascular system (24). What occurs then with this clot and what brings this process to be activated, is still not known.

2. BED REST AND IMMOBILISATION

It is known that bed rest has been a treatment strategy for many disorders since the early phases of medicine development, started with Hippocrates (25). But this process, sound at first gently, has shown to correlate with a lot of negative consequences to our body collectively referred as body deconditioning (26). This complex of the physiological changes, start to set within few days of immobility and can be explained independent of the basically disorders (27). Clinically, this fact enhanced the require of minimization as much as possible of the bed rest period and also special care during the time lying in bed, like frequent changes on position, passive and active range of motion exercises, adequate hydration and nutrition, maintains of continence, social contact encouragement etc. Physiological changes during bed rest immobilization have been found to occur also by space flight astronauts under the effect of microgravity. The relationship between these two conditions, enables the bed rest researches to provide data that are useful for the astronauts as well as people on earth undergoing prolonged bed rest immobilization (28).

Giving the lack of gravity a central role in this complex mechanism, most of the organ systems will be influenced, specially the cardiovascular, skeletal and muscular system (27). The muscles atrophy, their strength reduce 10-15% each week thus in 3- 5 week of immobility, undergo 50% strength loss (26). The antigravity muscles are the most involved into this process. A 119 days bed rest study, showed a volume decrease in the ankle plantar flexors (-30%), ankle dorsi flexors (-21%), quadriceps (-16), lower back muscles (9%)(29). Bones loss density and that leads in disuse osteoporosis. It is caused by an imbalance between resorption and formation of the bone mass. This is consequence of the lack of weight bearing as stimulus, as well as negative calcium balance exhibited by bed rest (27). The whole process mentioned above, increase the risk of fractures particularly to elderly, consequently prolonging the immobilization time (30). Deformities of the joints (contractures) have been also evidenced. This is result of remove and reorganization of connective tissue as well as shorten of collagen fibers (31).

Because counteracting gravity in an upright position is necessary for the optimal function of the cardiovascular system, it will be obviously influenced from the bedrest

immobilization. Maximal oxygen consumption (VO2max) decrease 0.9% over 30 days of bed rest (32). The mechanism that affect this decrease is showed on the Figure below.

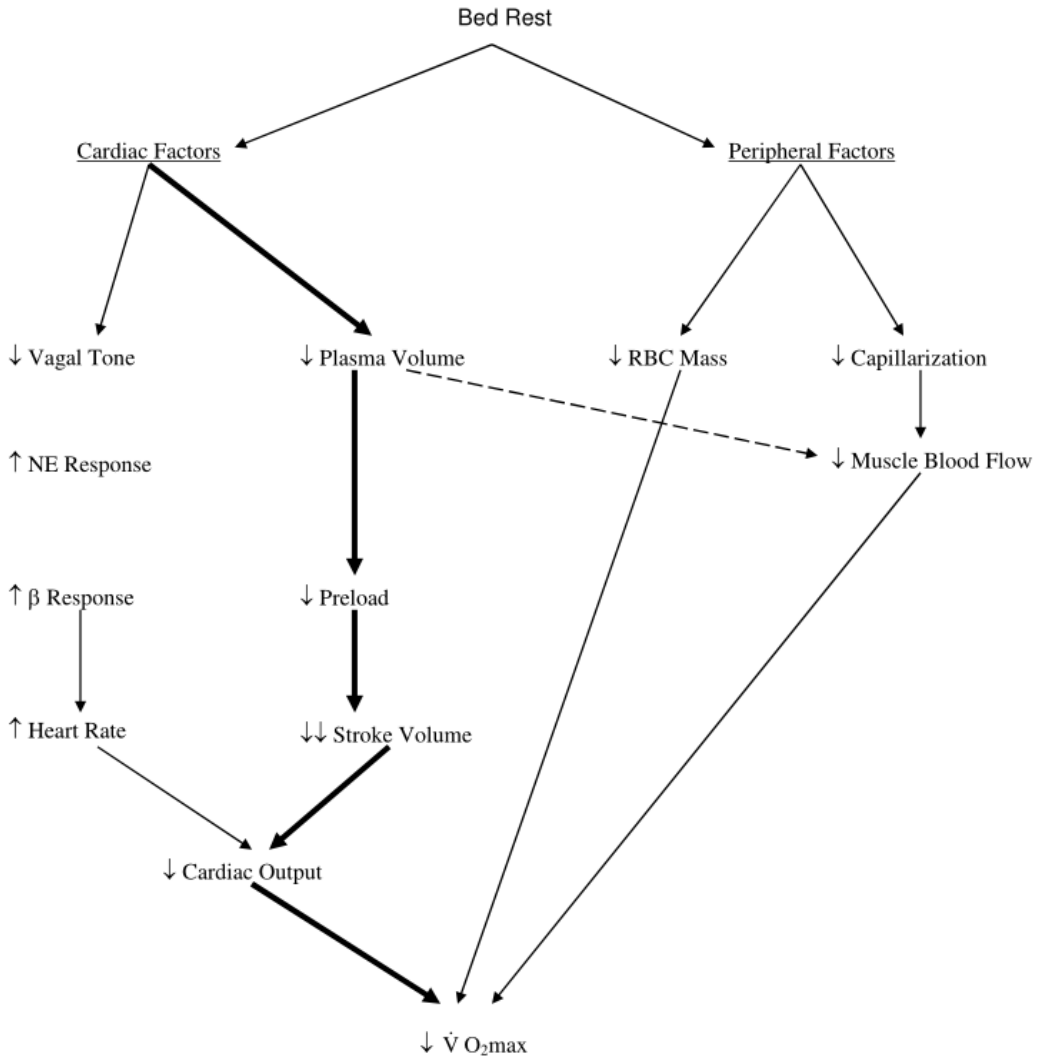

Figure 10: Mechanism that cause decrease of VO_2 as result of bed rest immobilization (27)

The laying position causes redistribution of body fluids (central shift) from the extremities to the thorax thus stimulating the release of ANP on the atriums and reducing release of the ADH from pituitary. The net result of these effects is diuresis as well as reduction of blood volume leading so to reduce of venous return (33). Also the loss of muscle strength contributes to venous reduction thus leading to reduce of stroke volume. The body counteracts this effect by gradually increasing the heart rate. It has been evidenced that after 4 weeks of immobilization, the heart rate in rest, increases by 10 beats pro minute, and by exercises 40 beats per minute (33). The increase of heart rate in rest is thought to

result also from decrease on vagal activity, as well as the contribution of noradrenalin release to the maximum heart rate have been also evidenced(27). Based on the principle ``use it or lose it``, heart is undergoing deconditioning resulting from myocardial atrophy (33). The deconditioning consequences of cardiovascular system, will affect the body also after remobilization thus leading to orthostatic hypotension. It occurs because the pooling of blood to the lower limbs when the person start to move after immobilization period, cannot be quickly corrected from the deconditioned cardiovascular system, and as result, the cerebral perfusion decrease, leading to dizziness and a tendency to fall(33).

Also the lungs are affected by immobilization periods, leading to reduce of the forced vital capacity (FVC) as well as forced expiratory volume in one sec (FVC1). The ciliary function is decreased thus increasing the risk of respiratory infections (33). Skin ulcers, constipation, atrophy of the intestinal mucosa and glands, urinary stone formation, mental disorders, loss of mineral and electrolytes, body fat increase, glucose intolerance are some other consequences of immobility.

It is supposed that hemostasis is also impacted from bed rest immobilization. The reduction of plasma volume will consecutive increase the blood viscosity, otherwise the atrophy of muscles, decrease the oxygen demand leading so to decrease of the number of red cells, their mass and hemoglobin concentration(33). A pathological condition that could be consequence of hemostatic dysbalance is formation of thrombus. To explain the mechanism of thrombogenesis, **the Virchow`s triad** is traditionally taken in consideration. This Virchow´s model, published in 1875, presented three components that lead to the formation of thrombus: abnormalities of vessel wall, abnormal blood constituents and blood flow abnormalities. The fact that abnormalities only on one component of the triad do not fully predicts hemostasis disorders, suggests primary for the complexity, interaction and multifactorial etiopathology of coagulopathies.

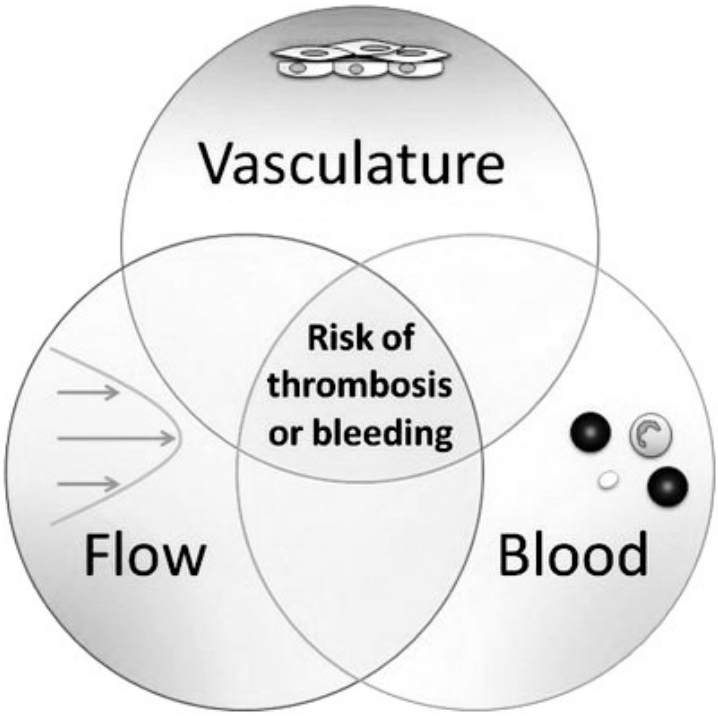

Figure 11: Elements of Virchow´s triad (34)

Increased prothrombin and fibrinogen levels, is known to be risk factors for thrombosis, at the other site, plasma factors deficiencies (like Factor VIII, IX) promote bleeding. Vascular wall elements are providers of a procoagulation surface as well as producer of signals that trigger coagulation (a central role into the process has tissue factor TF) (35).

Also the vascular wall *shear stress*, as a variable of the blood flow is showed to impact the thrombogenesis. Shear stress is the tangential force of fluid that act directly to the endothelium (35). It is the up regulator of Krüpel-like transcription factors (KLF) of endothelium, inducing so anti-inflammatory and antithrombotic effects. Based on the fact that immobilization leads to stasis, consequently reduce the shear stress which results on loss of KLF and enhanced the TF expressivity as a coagulation promoter component. Another key variable of the blood flow is wall *shear rate*, which describes the velocity that platelets and plasma solutes are delivered away from the vascular wall (radial distance) and then carried downstream (35). Shear rate is low by brunch points and curvatures, which correlate with regions of atherosclerosis. Also the increased pressure on the vascular wall, like seen on hypertension, is thought to increase the exposure of TF thus contributing to a procoagulant state (36).

Although the Virchow model primary referred to venous thrombosis, it can be used also for arterial thrombosis (37). Principally, the arterial thrombosis originates from rupture of atherosclerotic plaques, otherwise the venous thrombosis origin comes from plasmatic hypercoagulability in relationship with intact endothelium. This procoagulant activity appears to be triggered particularly by reduced blood flow, stasis, and inflammation as events vents that have been evidenced also on the persons undergoing bed rest immobility (35).

This relationship between bed rest immobilization and hemostasis is the focus of my literature research and is explored in this diplomarbeit.

II. AIMS AND OBJECTIVES

Based on the literature review, I will hypothesize what happens during bed rest immobilization with the coagulation. The primary question is how coagulation is affected by bedrest immobilization. As bedrested subjects are more labile to clotting, yet not all these subjects have clotting disorders, could it be possible that the increased pro-thrombotic state is balanced by an increased anti-thrombotic effect?

In addition, to searching for the past and current literature available on the web, this work will also focus on what state of the art markers of pro- and anti-thrombotic are available and how they are influenced by bed rest.

The effects of different postural changes as well as stress hormones and sitting immobilization on coagulation will be explored. The relationship to differences in methodologies, subject/ patient selection, types of interventions as well as differences in protocols are going to be discussed. This aspects is important to understand what really happens to the coagulation system in different physiological states (e.g. Immobilization, Stress).

The aim of the current Diplomarbeit is to provide an update on existing literature related to coagulation and bedrest immobilization. This work has application in the treatment and prevention of falls related thrombosis, bedrest immobilized subjects and the elderly.

III. METHODOLOGY

``Immobilization induced coagulatory changes´´ was the subject of my research.
To obtain systematically the relevant literature related to my topic, I constructed the search strategy into three main steps explained next.
At first I started using keywords that were identifying the essential subject (using free text terms): immobilization, hemostasis, microgravity effects, coagulation factors, publication immobilization. Then I looked for possible synonyms of these terms: ``immobilization``synonym``bed rest´´; ``hemostasis``synonym´´coagulation cascade´´, ´´blood clotting``. At last, I looked in Pubmed which of the terms mentioned above were there represented by using the MESH database. This strategy of searching in Pubmed as a database proper to my search enabled me to get most of the relevant papers for my search area. So, after finding the terms, I decided to explore the relevant publications.

Limiting the criteria of my research- Use of AND or OR or NOT helped me to focus on the field of interest and enabled me to get the publication that were appropriate for my research. The words combination that I used is: Immobilization AND coagulation; Bed rest AND coagulation; Blood clotting AND microgravity.
Another tool of helping me to specify the articles related to my research was putting of the keywords on the quote (").

Refining my search criteria- Because my research topic is specifically related to the aspect of pathophysiology of the coagulation and the immobilization effects on it, obtaining all the publication that have to do with coagulation and immobilization was not only waste of time and annoying for my work but also I could not get the publications that were appropriate for my research (to many articles found). So, to avoid the unnecessary publications I refined my search criteria by using also the word NOT:
(coagulation NOT coagulation, treatment) AND immobilization.
Another element placed on this field was the language of the publications (I choose English and German languages). By the publication dates field I defined the last 57 years (1957- 2014) as another criteria for my research.

The sources of my research: **PubMed** was the primary database chosen from me. It not only contains the publications related to my topic but also is very good updated and has a

large number of publications over the years. It is also simple to use, you can read many of its articles as volltext and it makes a list of the last publications recently seen. But even though PubMed is what I use most I could not find every article I wanted. Therefore, I used **Web of Science**. This database found I particularly good what the information of the authors concerns. Also, seeing how many times one paper has been citing, found I practice in finding the articles related to area of search and also most of the citing articles are newer that the original articles.

The list of References was also very helpful for my search even when the original article was not enough appropriate for my topic, so sometimes the references of one article were the publications that I was looking for**. Google** used I particularly by searching for pictures and the information included on the introduction of my work.
Choice of the literature- I selected the literature based on these criteria:

1. Was it relevant to my topic?
2. The articles that i looked for were written in the last 57 years
3. Abstracts plus full texts were included as primary literature
4. Books particularly physiology and pathophysiology books as well google were used as secondary literature
5. Only articles written in English or German were used.

Organizing the references selected/ used for this work- I used Refworks to save the references. So, I imported these directly from the Pubmed or Web of science to my Refworks account, having also the opportunity to format them.

IV. UPDATE OF THE LITERATURE

This section of the DA is divided into three categories:

A) The data provided from the first studies being researched, offer different view perspectives of the relationship between hemostasis and bed rest. Some of the studies done on the bed rested subjects found no clinically significant changes of the hemostatic parameters on the persons confined to bed while the others associated bed rest with thrombosis development and yet the others showed decrease of the hemostatic parameters F1+F2, TAT, decrease of PAI-1 as well as down regulation of PGDF and P- selectin markers.

B) Explored in the current literature are also the studies focused on sitting immobilization and its effects on hemostasis.

C) The final aspect of the DA examines the studies that explored the effects of different postural changes as well as stress hormones on coagulation. The relationship to differences in methodologies, subject/ patient selection, types of interventions as well as differences in protocols are going to be discussed. This aspects is important to understand what really happens to the coagulation system in different physiological states (e.g. Immobilization, Stress, etc.)

A) Studies dealing primarily with bed rest

Table 3: Bed rest studies

Investigators	Subjects (n)	Bed rest length/model	Results
Gibbs et al	238 dead persons	till 12 weeks/supine position	↑incidence of thrombosis
Rosenfeld	12 mixed gender	36 hours/supine position	↑fibrinolysis ↓ PAI-1 ↑Cortisol early morning Catecholamine no change
Heider et al	24 men	60 days/ 6° HDT	Non-significant
Waha et al	12 men	21 days/ 6° HDT	Non-significant
Arinell et al	15 women	60 days/ 6° HDT	↓PGDF,↓P-selectin

		20 days recovery	↓platelet act/aggr. Values remained ↓
Broderick et al	10 (6 men and 4 women)	4 hours	Venous stasis significant
Colucci et al	10 men	30 min./pressure cuff around the thigh	↓F1+F2

Now let me discuss the above mentioned (Table 3) studies in detail.

Gibbs (38) explored the lower limbs venous thrombosis referenced particularly on bed rest. The data from 239 dead patients that in the course of their hospitality (till 12 weeks) developed venous thrombosis were analyzed. Based on their primary illness that caused their death (cardiovascular, cerebrovascular, trauma, suicide, medical, surgical) they were classified into classes. Also the duration of bed rest served as criteria to categorize these patients into groups. The incidence of venous thrombosis showed to increase progressive as the duration of bed rest increase, beginning after 2-3 days of bed confinement by traumas and affecting fast all the patients after one week. Increasing bed rest was associated with progressively increase of the thrombus age; this contradicted the known belief that thrombosis leads to imminent death.

Rosenfeld and his colleagues (39) explored the effects of 36 hours of bed rest on the circadian changes (measured at 8 am, 10 am, 4 p.m., 8 a.m.) in twelve healthy volunteers. Except the coagulation parameters including platelet activation, also the neuroendocrine hormones (cortisol, epinephrine and norepinephrine) were analyzed. The only hemostatic parameter that significantly changed was the plasminogen activator inhibitor 1 (PAI-1), which decreased over time. Cortisol showed an early morning increase; catecholamine, on the other hand, showed no changes. This result indicated no procoagulatory effects caused from the bed rest. Indeed an enhanced fibrinolysis was evidenced, thus suggesting a protective counter effect of the body to the venous stasis observed in bed rested subjects.

Years later, **Haider and his colleagues** *(40)* examined the effects of long-term head-down-tilt bed rest, and different training regimes, on the coagulation system of healthy men. Their study was based on 60 days of prolonged bed rest of 24 healthy men under simulated microgravity (6 degree head down-tilt). Three groups of bed rested subjects were

examined: only bedrest, bedrest + resistive exercise only and bedrest + resistive exercise with whole body vibration group. The results of the study showed the same dynamic pattern of hemostatic parameters (DD, TAT and PT F1+2). At the first day of the bed rest, the values decreased. Then within the next four days an increase was shown (peak at day 5), following from a slight dropping till the end of the bed rest, then a decrease in the reambulation period (peak day 3). Although these changes were evident in the study, none of the coagulation parameters reached a plasma concentration that may indicate any thrombotic state. Also the ROTEM parameters showed significant changes over time but they remained within the normal range thus no pathological traces were found. The different training modalities did not have any influence on the parameters measured on the study. They concluded that there is no clinically significant change on the hemostasis parameters during the 60 days bed rest both in the prothrombosis and/or fibrinolysis. Furthermore, the training interventions did not influence the hemostasis.

No evidence of procoagulatory as well as anticoagulatory effects by persons undergoing medium term bed rest is found also on the study done by **Waha et al (41).** They explored the effects of 21 days of bed rest (6^{o} head down model) in 12 healthy men. Thrombelastometry and poor platelet plasma were used to collect the data. A prolongation of the coagulation time during the whole study was significant, although a high standard deviation is showed from the ROTEM. α-angle values, depicting the availability of fibrinogen in plasma, were reduced, indicating a lower tendency to clot. But, none of these parameters showed a pathological values, which would indicate hypercoagulability. F1+2 did not show a procoagulatory values in study time, in fact a significantly decrease was noticed on day seven and day twenty-one during the bed rest period. A decrease on second day after recovery showed prothrombin, without changes along the bed rest. A continual reduction of endogenous thrombin potential (ETP), which could be indicative of a reduction of general thrombin formation, was observed. Other thrombin generation parameters showed also significant changes, but without reaching pathological values. Furthermore, some other blood parameters (TAT, t-PA, factor VIII) were also analyzed but no significant changes were observed. Despite some significant changes being evidenced, all the measured parameters were in the normal range. This study, therefore, concluded that medium term bed rest immobilization does not shift the hemostasis neither to a pro- or anticoagulatory state.

Furthermore, **Arinell and his colleagues** (42) explored the effects of long-term bed rest immobilization on platelet function. They investigated the changes of platelet-derived growth factor (PDGF) and P-selectin as markers of platelet activation. 15 healthy women underwent 60 days bed rest in head-down tilt (6 degree) and some of them did aerobic exercises while confined to bed (active group) while others did not. The results of the study showed that compared to the baseline, PGDF and P selectin levels decreased after bed rest and they remain decreased also in 20 days recovery. The number of platelets did not change and no differences were found between inactive and active group. Based on the results of the study, a down regulation of platelet activation during long-term bed rest period was suggested.

Venous stasis is considered to be an important risk factor incorporated on thrombogenesis. Does four hours bed rest on lower limb blood flow by healthy persons cause venous stasis? **Broderick and his colleagues (43)** investigated this**.** One group received an intense electrical stimulation around calf musculature while the other did not. The results showed that in the non-stimulated group, a decline of 45% popliteal venous blood flow was evidenced whereas in the stimulated group a higher maintenance of blood flow was seen thus leading them to conclude that even 4 hours of bed rest could lead to venous stasis**.**

In a later study, **Colucci and his colleagues (44)** investigated the possible effects of venous pooling on coagulation. They provoked venous stasis artificially in ten healthy men without risk factors for thrombosis. A pressure cuff around the thigh (50 mmHg inflated) was applied for 30 min after 15 min of rest. Although the measured blood values remained within normal range, decrease of plasma F1+2 was significant, suggesting an association between decrease of F1+2 and venous stasis. Whether this decrease is associated with prothrombosis is still hypothetical: As thrombin is known to promote also protein C pathway by an intact endothelium as well as by activating TAFI, down regulation of its generation could lead to decrease of protein C and TAFI. This could destabilize the pro- and anti-coagulation equilibrium.

B) Studies that compared the effects of different body postures as well as stress hormones on coagulation

Table 4: Studies with different body postures and coagulation

Study done by	Study models	Results
Brezinski et al (n=16)	St.1: normal activity till 12^{30} - 8 am waking up but lay on bed - 9.30 am upright posture -11 till 12^{30} ambulatory St.2: bed rest till 12^{30}	 Non-significant ↑ Platelet activity/aggregation ↑ catecholamine, renin, AT II; Non-significant Non-significant
Zaar et al (n=10)	10min exposure on 30mmHG LBNP	↓SV, ↓CO, ↓MAP ↑TAT, ↓aPTT slightly, ↓plasma pancreatic polypep. ↓Vagal activity
Wirtz et al (n=42) Relationship between MBP and catecholamine procoagulant activity	Normotensive and mild hypertensive -rest period -stress period -recovery period	 Pro-coagulant Relation by hypertension. Non- significant Pro-coagulant Relation by hypertension. Non-significant relation with cortisone levels on all situations and groups
Känel et al (review)	15-40 min epinephrine infusion	↑procoagulatory effects ↑fibrinolysis ↑Platelet activation
Liverani et al	Prednisone effects on coagulation	↑ inhibition of platelet adhesion
Masoud et al (n=19)	- supine position -15 min of still standing	3 subjects ↓PCAT-NR; ↓PCAT-NR,↑TF, ↑Prot.S Ag,

		↑Prot.C;
	-30 min of still standing	↓Plasma volume, ↑Plasma proteins,↑D-dimer,↑vWF, ↑fibrinogen;
	-60 min of still standing	↑F1+F2 steeply, ↑FVa, ↑FVIIIa, ↓aPTT;
Cvirn et al (n=7)	-Graded Orthostatic stress	↓25% of plasma volume ↑F1+F2, ↑TAT ↑TF, t-PA
	-Recovery	↑F1+F2 ↑TAT
Venemans-Jellema et al (n=49)	-WISE study: 24 women, 60 days/6º HDT -Concordia study: 25 men, hypobaric hypoxic conditions	On both studies: ↓F1+F2;↓TAT
Kristensen et al (n=100)	- normal activity -bed rest	No statistical evidence of renew hemorrhage risk on both groups
Radl et al (n=100)	-Mobile post-op patients without prophylactic thrombosis medication	Four older patients developed thrombosis

Now let me discuss the above mentioned (Table 4) studies in detail.

Another perspective of coagulation was provided by **Brezinski and his colleagues** (45), who explored the effects of morning activities on platelet aggregability. 16 healthy persons took part on the study, in a randomized cross-over design in which they did either lying in bed till 12.30 pm (control) or waking up at 8.00 am, then lying in bed until 9.30 am and then assuming an upright posture until 11 am and then this was followed by an ambulatory period of one and half hours in which they did normal daily activities. The results of the study showed an increased platelet activity and aggregability on the time between 9.30 and 11 AM. This suggests that the assumption of upright posture in the morning increases platelet aggregation. Changes of posture were also associated with increases in epinephrine, norepinephrine, angiotensin II as well as renin. On the control day, no significant changes on platelet aggregability as well as plasma epinephrine were observed.

These effects include not only the catecholamine release and vasoconstriction, but also the increase of platelet aggregability.

These platelet activity and aggregability changes seen during postural changes are also seen in simulated acute hemorrhage *(46)*. **Zaar and his colleagues** *(46)* used the lower body negative pressure (LBNP) model to simulate central hypovolemia. Their hypothesis was that the coagulation markers would increase by moderate decrease of central blood volume (CBV) during LBNP. To test this, 10 healthy men were exposed 10 min of 30 mmHg LBNP. The procoagulant plasma markers measured by the study are: thrombin-antithrombin III complex (TAT), D-dimer, Prothrombin time (PT) and activated partial thromboplastin time (aPTT). The cardiovascular variables as well as plasma pancreatic polypeptide were also evaluated. The last parameter is used as indicator for the parasympathetic activity thus investigating also the relation between parasympathic system and coagulation activity by LBNP exposition. The results of this study has shown that during LBNP, heart rate did not change but the stroke volume, cardiac output and mean arterial pressure were decreased. From the plasma markers, significant was the increase of TAT complex (5 fold after 5 min of LBNP), indicating so the activation of the thrombin part coagulation systems during the influence of LBNP. Furthermore, D-dimer plasma was unchanged, indicating that despite thrombin increase, no stable clot formation has been provoked. PT did also not change; aPTT decreased slightly showing that the coagulation factors have not been consumed despite the thrombin increase. The plasma pancreatic polypeptide decreases thus indicating decrease of vagal activity, therefore suggesting that parasympathic activity has not been responsible for the plasma TAT increase. In summary, the study concluded that the reduction of CBV induced from LBNP as simulation of a mild to moderate hemorrhage, activates hemostasis by increasing the TAT complex not related to vagal activity.

Wirtz and her colleagues (47) investigated the role of hormones in hemostatic processes. They suggested that epinephrine (associated with FVIIIa levels) and noradrenaline (associated with fibrinogen levels) activity are part of the regulatory system important for maintaining a steady coagulation state. Furthermore, they explored the relationship of mean blood pressure with procoagulant activity of the catecholamines at rest as well as during acute stress and recovery period. 42 healthy subjects (free of medications), normotensive and mildly hypertensive, took part on the study. The results showed interaction between mean blood pressure (MBP) and catecholamine for coagulation at rest,

while during stress or recovery only a borderline significance was evident. So, in persons with mild hypertension, catecholamine showed a significant relationship with D-dimer increase and FVII activation. In normotensives, this relationship was not so intense. Independent of MBP, age and BMI showed no impact on procoagulatory catecholamine activity. During stress, effects of hormones on coagulation seemed not to be related with MBP. Similarly, there was no relationship between cortisol and procoagulation at rest or during acute stress. It is possible that hormonal activity may play a role in coagulation during recovery and therefore there is a need to screen the MBP as well. Further studies are needed to examine this.

Short-term sympathic effects on hemostasis reviewed from **Känel et al**, suggest a dose-dependent hemostatic stimulation within 15-40 min of epinephrine infusion. Stimulation of adrenoreceptors (mainly ß2 and α2) via catecholamine infusion release coagulation as well as fibrinolysis factors, and activates platelets. The procoagulatory effects of short term SNS effects, is thought to be particularly important in persons that already have risk factors for thrombogenesis like atherosclerosis, hypertension etc. In healthy persons this may not present a problem.

Direct effects of glucocorticoids on hemostasis were suggested by **Liverani et al (48).** They observed the inhibitory effects of prednisolone under static conditions on platelet adhesion and spreading on collagen as well as the inhibition of thrombogenesis under flow conditions. To achieve these effects, prednisone needs activation mediators of the thrombocytes (platelet ADP, TxA). This suggests that glucocorticoids appear to act via activation dependent components of adhesion and aggregation.

Masoud el al (49) explored the orthostatic stress and its relationship with hemostasis. From 19 healthy persons, age between 21 and 45, coagulation/anticoagulation parameters, plasma shifts and plasma proteins were measured. At first were analyzed the data from supine rest, then at 15 min, 30 min and 60 min of still standing. At 30 min standing, a decrease of plasma volume and increase of plasma proteins was observed. Factor V, Factor VIII, fibrinogen were increased as well as a increase of prothrombin fragment 1+2, tissue factor (increase after 15 min of still standing) von Willebrand factor and D-dimer were evident. On the other hand, anticoagulation factors, mainly protein C pathway (free protein S und Protein C), were activated but they decreased continuously as standing progressed and also the global activity of the protein C pathway decreased significantly (PCAT-NR

measure). The last result is thought to be a consequence of Factor VIII and Factor V increment thus overwhelming the inhibitory capacity of the protein C pathway. In summary this study showed that a hypercoagulability state occurred during orthostasis (standing up). Furthermore this study suggests that there may be a possibility that some other mechanisms such as activation of sympathetic system, renin-angiotensin system, and vasopressin activation, all could promote the hypercoagulability state.

Also **Cvirn and his colleagues** (50) speculate the involvement of endocrine system particularly ACTH, vasopressin and catecholamine as well as a generalized stress response to occurrence of a hypercoagulability state during standing up leading to presyncope. They explored the effects of graded orthostatic stress on hemostasis. They used a combination of head up tilt (HUT) and LBNP to provoke presyncope on 7 healthy males. The blood samples collected during different times of the study showed a loss of 25% plasma volume at peak of orthostatic loading. During the protocol significant was the increase of prothrombin, thrombin, endogenous prothrombin potential and tissue factor pathway inhibitor and blood cell counts analog to hemoconcentration. Differently reacted, however, the thrombin generation indicators (F1+2, TAT) and endothelial activation factors (TF, t-PA), which increased far beyond the effect of hemoconcentration. At the 20 min recovery period almost all of the coagulation factors returned to baseline supine values except F1+F2 and TAT, which remained increased also during the recovery period, suggesting an increase in coagulation. The hypercoagulability observed during recovery may predispose to thrombogenesis in high risk persons. Because shear stress is terminated at recovery, persistence of increased thrombin generation factors indicate the exist of other mechanisms involved on this hypercoagulable state.

Another study done by **Venemans-Jellema and her colleagues (51)** aimed to elucidate the separate effects of hypobaric hypoxia and prolonged immobilization (both hypoxia and immobilization are considered as risk factors for thrombogenesis by the persons that undergo air travel) on the coagulation system. Whether one of these or both of these are necessary for thrombus formation was the core issue of the study. The first study (WISE study) was done on the 24 healthy, nonsmokers, adult women that underwent 6^{o}head down bed rest for 60 days. This group was divided into three subgroups: only bed rest, bed rest plus exercise countermeasure, bed rest plus nutritional countermeasure group. In the second study, carried out the ESA facilities isolation station at Antarctica (Concordia station, http://en.wikipedia.org/wiki/Concordia_Station), which is an hypoxic hypobaric

environment due to its high altitude location, blood samples of 25 healthy men, who sojourned there for one year were taken for analysis. In both the studies, compared with baseline values, no evidence of increased coagulation markers was found. Instead of it, during bed rest as well as during exposure on hypoxic environment, a decrease on F1+F2 measurement, compared with values before and after the studies states, is being evidenced. Also the same pattern is evidenced for TAT values although the decrease level was less explicit. The measure that did not change over time was the D-dimer level. In compare of the results between three subgroups of the first study, came out that the F1+F2 level were higher in the exercise subgroup than in other subgroups. So, the net result of the study showed no coagulation activation by healthy persons undergoing bed rest as well as prolonged hypoxia. The higher level of F1+F2 on the exercise subgroup is explained to be as a result of muscle movements, which is likely to trigger the activation of a minute coagulation to a constant level. The results of the hypoxic and the bed rest studies indicated that the immobilization per so by healthy persons does not induce thrombin formation. Furthermore, they suggested that on the air flight a combination of risk factors is required to form thrombus, not only a separate factor like hypoxia or immobilization **(51)**.

Kristensen VG and his colleagues (52) explored the effects of mobilization vs bed rest on the risk of renewed hemorrhage in patients with primary epistaxis. 100 participants were divided into a mobile group and control bed rest group. The results showed no statistically significant evidence that bed rest reduces the renew hemorrhage risk or that the mobilization claims it. Clinical significance of the study is that there may not be a need of bed rest treatment in persons with primary epistaxis. This would prevent the deconditioning complications of bed rest and facilitate benefits to patients being mobile during their stay in the hospital.

Radl and his colleagues (53) explored the rate of venous thrombosis in postoperative patients after hallux valgus surgery. In the study were included the data of 100 subjects (mean age 48.9 years) that did not take prophylactic anti-thrombotic medication, and who were without risk for thrombogenesis. Moreover, 21 of them were taking contraceptives. The subjects were assessed with phlebography 29 days after surgery. They were mobilized since the first day postoperatively and dressed below the knee compression stockings only at the time staying in hospital. In four subjects, a thrombus development was found. The mean age of these four subjects was greater than 61.7 +/- 6.1. So, based on the results, this

study suggests that there is no need for routine medical prophylaxis to all the patients undergoing a valgus surgery. Furthermore, prophylaxis should be calculated for each patient according to the individual risk factors and in patients over 60 years routine prophylaxis should be used.

C) Studies that focused on sitting immobilization and its hemostatic effects

Table 5: Studies dealing with hemostasis and sitting for longer periods

Study done by	Subjects	Model	Results
Stricker et al (n=58)	n=40 n=18	Sitting for 6 h Free move for 6h	↓F1+F2 Non-significant
Schreijer et al (n=71)	30n- no risk factors 11n-factorV Leiden mutation 15n- oral contraceptive use 15n- both of the risk factors	All subjects underwent: 8h air flight 8h movie marathon 8h daily life activity	↑TAT,↑F1+F2, ↑D-dimer ↓TAT ↓TAT
Schobersberger et al (n=20)	10n low-no RF 10n moderate RF	Long haul flight (wien-washington-wien)	To all subjects: ↑FVII, ↑FVIII ↓fibrinolysis
Schobersberger et al (n=19)	19n	Bus trip 10h (measures: before, during, after the trip)	After the trip: ↑procoagulatory effect, ↑F1+F2 ↑leg volume ↓t-PA ↓t-PAI1

Now let me discuss the above mentioned (Table 5) studies in detail.

Stricker et al (54) aimed to test the hypothesis that the activation of coagulation occurs in persons undergoing long-term immobilization in sitting position such as in air flight. The first group contained 40 healthy subjects with average age 29.5years, which were seated for 6 hours and a control group of 18 subjects, which moved freely. The results of the study showed no change on the fibrinopeptid A and DD level, whereas the F1+F2 levels

decreased over the 6 hours immobility period. In the control group in sitting position, F1+F2 decrease was evidenced, whereas no changes were seen under ambulatory conditions. Therefore, no thrombin generation due to long term sitting position could be observed: the decrease of F1+F2 (suggesting down regulation of hemostasis) could indicate a protective mechanism to counteract venous stasis, which occurs during sitting.

Separating the air flight effects from immobilization and circadian rhythms by subjects with and without risk factors was the aim of the study done by **Schreijer et al (55).** They used the data taken from 71 healthy volunteers, who 30 of them had no risk factors, 11 had factor V Leiden mutation, 15 subjects took oral contraceptives and the last 15 had factor V Leiden Mutation as well as took oral contraceptives. All Persons participated in three different exposure situations: 8 hours air flight, 8 hours movie marathon (sitting position immobilization) and 8 hours daily life. A median concentration increase on TAT complex after air flight is evidenced, contrary to immobilization and daily life measures, who the TAT complex median concentration decreased. Furthermore, the levels of TAT after flight is recorded to be higher (11 of 66) in compare with movie marathon (2 of 68) and daily life (1 of 70). The changes were most evident by the group that took oral contraceptives and had factor V Leiden mutation. A high response on F1+F2 and D-dimer is evidenced only by the persons after flight.

Schobersberger and his colleagues (56) evidenced also the activation of coagulation during a long haul flight (Wien- Washington). Twenty persons took part on the study. Ten of them presented no till low risk factors for VTE, the other ten were identified as moderate risk persons to develop VTE. The results of the thrombelastographic measurements showed activation of the coagulation to all the subjects, Factor VII and factor VIII were significantly increased and fibrinolysis suppressed, without differences between two groups.

A moderate activation of the coagulation system is also found by another study done from **Schobersberger and his colleagues (57)**. Explored were the hemostatic changes during a long-distance bus travel. Data from 19 healthy subjects who undergo a 10 hours bus trip were analyzed. Also the volume changes on the legs were measured. Thromboelastography results showed a moderate activation of the coagulation, as well as an increase in prothrombin fragment 1+2 was evidenced. Furthermore, significant was a decrease of t-PA and t-PAI-1 as well as an increase on leg volume mainly localized in the calf. The results

of the above mentioned studies suggest existing of additional mechanism to immobilization that is incorporated on thrombogenesis by air flight traveling, and also underline the importance of individual risk factors on the hemostatic changes.

V. SUMMARY AND FUTURE DIRECTIONS

Bed rest immobilization has been suggested to affect coagulation. However, based on the studies that were explored in this DA, there appears to be no consensus on the reported data. Some studies did not found any significance, while the others reported a down regulation of hemostatic activity due to immobilization. Evidence of an enhanced fibrinolysis as well as a down regulation of platelet activation during bed rest suggest a protective counter effect of the body to the venous stasis observed in bed rested subjects. Furthermore, decreases in F1+F2 were also reported. Whether this decrease is associated with prothrombosis is still hypothetical: As thrombin is known to promote also protein C pathway by an intact endothelium as well as by activating TAFI, down regulation of its generation could lead to decrease of protein C and TAFI. This could destabilize the pro- and anti-coagulation equilibrium.

On the other hand, assumption of upright posture in the morning has been reported to increases platelet aggregation. Does it imply that it is not the bed rest per se but perhaps the assumption of upright posture by previously immobilized persons which causes a hyper clotting state? The answer needs furthermore explorations.
Similarly, a hypercoagulable state is also reported during orthostasis (standing up). This is believed to arise due to activation of sympathetic system as well as to the accompanying shear stress. However, the hypercoagulability reported also during the recovery period may indicate existence of other mechanism involved in this hyperclotting state that may predispose to thrombogenesis in high-risk persons. In healthy persons this may not present a problem.
However, the pro-coagulatory effects of catecholamine is thought to be particularly important in persons that already have risk factors for thrombogenesis like atherosclerosis, hypertension, elderly etc.

In summary, the explored studies in this DA suggest that bed rest immobilization per se does not induce pro-coagulatory changes. This statement underline the importance of individual calculation of risk factors on the hemostatic changes during bed rest immobilization.

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