Diplomarbeit

MATERNAL AND FETAL OUTCOME OF PREGNANCIES WITH FACTOR V-LEIDEN – A RETROSPECTIVE ANALYSIS

eingereicht von

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Graz, am 15. Jänner 2015 *Dijana Jagic eh*

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Abstract:

Objective: Factor V Leiden- Mutation is an autosomal-dominant inherited defect of the coagulation cascade. It affects about 5% of the population. Compared to the healthy population, heterozygous individuals have a 5- to 10-fold increased risk and homozygous individuals a 50- to 100-fold increased risk for thromboembolic events. Thromboembolisms are often manifested when additional risk factors occur, for example during pregnancy. Aim of this study is to evaluate maternal and fetal outcome of women with factor V Leiden-Mutation dependent on the therapy that women have received during pregnancy.

Patients and methods: We reviewed data of 104 pregnant women with Factor V-Leiden Mutation, who were cared throughout pregnancy and delivered in the years 2006 and 2012 at the Department of Obstetrics of the Medical University of Graz. We divided our study collective (n=104) into two groups: *1st group:* patients, who received antithrombotic therapy (n= 82, 78.85%) and *2nd group:* patients, who were not treated (n=22, 21.15%). Furthermore, depending on the therapy that was applied during current pregnancy, we divided the group of treated women into 3 groups: (1) women, who received LMWH (n=78, 95.12%), (2) women, who received only low-dose aspirin (n=1, 1.21%), and (3) women, who received both of these antithrombotic drugs (n=3, 3.65%). Based on pregnancy complications that were noted, maternal and fetal outcome was evaluated.

Results: *1st group:* Maternal outcome: 24 women (29.27%) developed pregnancy complications. The most common pregnancy complication was pre-eclampsia (n=5, 55.6%). Fetal outcome: 8 pregnancies (9.76%) were associated with IUGR. Whereby, 5 cases (62.5%) were associated with LWMH, one case was associated with low-dose aspirin, and the remaining 2 cases (25%) were associated with administration of both LWMH and low-dose aspirin. *2nd group:* Maternal outcome: 3 women (13.64%) have developed pregnancy complications. Whereby, the case of pregnancy-related venous thromboembolism (n=1, 33.37%) was relevant for our study. Fetal outcome: 3 cases (13.64%) of fetal complications were documented. IUFD appeared only once (33.33%) and the remaining two cases were IUGR (66.67%).

Conclusion: The usage of antithrombotic drugs improves maternal outcome, but it has a negative impact on fetal outcome. Further studies should be conducted in order to evaluate the necessity for adjustment of the antithrombotic therapy in women with diagnosed FVL-Mutation.

Zusammenfassung:

Fragestellung: Faktor V-Leiden ist ein autosomal dominant vererbter Defekt der Gerinnungskaskade. Etwa 5 Prozent der normalen Population sind davon betroffen. Die Mutation ist in der heterozygoten Form mit einem fünf- bis zehnmal, in der homozygoten Form mit einem 50- bis 100mal höheren Thromboserisiko verbunden. Thromboembolien manifestieren sich häufig beim Vorliegen zusätzlicher Risikofaktoren, wie zum Beispiel während der Schwangerschaft. Ziel dieser Diplomarbeit war, abhängig von der Therapiemodalität das mütterliche und fetale Outcome bei Patienntinnen mit bekannter Faktor V Leiden-Mutation zu evaluieren.

Patientinnen und Methoden: Diese Studie schließt 104 Patientinnen ein, die die Faktor V-Leiden Mutation aufweisen und in den Jahren zwischen 2006 und 2012 auf der Klinik für Geburtshilfe der medizinischen Universität Graz im Rahmen einer Schwangerschaft betreut und entbunden wurden. Es wurden zwei Gruppen gebildet. Gruppe 1 (n=82, 78,85%) inkludiert alle Patientinnen, die während der Schwangerschaft eine antithrombotische Prophylaxe erhielten und Gruppe 2 (n=22, 21,15%) enthält alle Patientinnen, die während der Schwangerschaft keine antithrombotische Therapie einnahmen. Des Weiteren wurde die Gruppe der behandelten Patientinnen abhängig von der Therapie in 3 Subgruppen unterteilt: (1) Patientinnen, die NMH erhielten (n = 78, 95,12%), (2) Patientinnen, die nur Aspirin erhielten(n = 1, 1,21%), und (3) Patientinnen, die diese beiden Antithrombotika (n = 3, 3,65%) einnahmen. Basierend auf Schwangerschaftskomplikationen, die erfasst wurden, wurde sowohl das mütterliche als auch das kindliche Outcome evaluiert.

Ergebnisse: *Gruppe 1:* Mütterliches Outcome: 24 Patientinnen (29,27%) entwickelten Schwangerschaftskomplikationen, wobei die am häufigste vorkommende Schwangerschaftskomplikation die Präeklampsie war (n=5, 55,6%). Fetales Outcome: 8 Patientinnen (9,76%) erlitten eine IUWR, davon erhielten 5 (62,5%) NMH, eine wurde mit Aspirin behandelt und die restlichen 2 Patientinnen (25%) erhielten sowohl NMH als auch Aspirin als Prohylaxe. *Gruppe 2:* Mütterliche Outcome: 3 Patientinnen (13,64%) entwickelten Schwangerschaftskomplikationen, wobei nur der eine Fall des thromboembolischen Geschehens (33.37%) für diese Studie relevant war. Fetales Outcome: 3 Fälle (13,64%) fetaler Komplikationen wurden dokumentiert. IUFT erschien nur einmal (33,33%) und die restlichen 2 Fälle (66,67%) waren IUWR.

Schlussfolgerung: Die Verabreichung von antithrombotischer Therapie hat zwar einen positiven Einfluss auf das Auftreten von mütterlicher Komplikationen, aber sie hat auch eine negative Auswirkung auf das Auftreten von fetaler Komplikationen. Um auf die eventuelle Notwendikeit einer Therapieanpassung bei Patientinnen mit bekannter Faktor V Leiden-Mutation hinzuweisen, wäre Durchführung weiterer Studien sinvoll.

Glossary:

APC	Activated protein C
APCR	Resistance to activated protein C
Anti-β2GP1	Anti-beta 2 glycoprotein 1
Anti-CL	Anticardiolipin antibody
aPL	Antiphospholipid antibodies
APS	Antiphospholipid syndrome
AT	Antithrombin
CI	Confidence interval
CNS	Cenral nervous system
F	Factor
FVL	Factor V Leiden
GDM	Gestational diabetes mellitus
HB-EGF	Heparin-binding epidermal growth factor
HELLP	Hemolysis, elevated liver enzymes, low platelet count
HIT	Heparin inducted thrombzytopenia
HRT	Hormone replacement therapy
IUFD	Intrauterine fetal death
IUGR	Intrauterine growth restriction
LA	Lupus anticoagulant
LMWH	Low-molecular-weight heparin
MTHFR	Methylene tetrahydrofolate reductase
OC	Oral contraceptive
OR	Odds ratio
ORs	Odds ratios
PC	Protein C
PIH	Pregnancy induced hypertension
PmC	Placental-mediated complications
PS	Protein S
SGA	Small for gestational age
SLE	Systemic lupus erythematosus
TF	Tissue factor
TFPI	Tissue factor pathway inhibitor

UFH	Unfractionated heparin
VTE	Venous thromboembolism
WHO	World Health Organization

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1. Introduction

1.1. Physiology of Blood Coagulation

Hemostasis (or haemostasis) is the process which protects our body from bleeding [1,2,3,5]. Hemostasis can be activated by any kind of blood vessel injury, which causes the destruction of their layers. It is achieved by several events: vascular constriction; platelet aggregation – formation of a platelet plug (or primary hemostasis); blood coagulation – formation of a blood clot (or secondary hemostasis). Vessel repair, clot restriction and dissolution complete this healing process [2,5].

1.1.1. Primary Hemostasis

Primary hemostasis begins with a vascular contraction in the area of the injured blood vessel. The degree of vascular spasm increases proportionally to the degree of the vessel trauma. An injured blood vessel can be healed by a platelet plug, or through a blood clot. It depends on the degree of the injury and on the size of the injured blood vessel. Primary hemostasis lasts until the injured area is sealed with one of these clots [2].

When a blood vessel wall is damaged, endothelial cells on the vessel surface are also destroyed. This uncovers and exposes the subendothelial collagen fibers to circulating blood [4,5]. Mediated by the von Willebrand Factor (plasma adhesive protein), platelets begin to adhere to the walls of the damaged vessel and to each other. Platelet aggregation leads to the formation of a platelet plug [4-7]. This also called loose plug is able to stop minor bleedings [2,5], but in most cases it has to be stabilized through a blood clot. This procedure can last from a few seconds up to a few minutes [4].

1.1.2. Secondary Hemostasis

Secondary hemostasis is activated simultaneously to primary hemostasis [7]. In case of severe blood vessel trauma, the blood clot begins to develop in 15 – 20 seconds. In case of minor injuries, hemostasis is activated after 1 – 2 minutes [2,4]. The conversion of fibrinogen, which is circulating in plasma into fibrin represents the most important reaction in the mechanism of blood coagulation [2]. Fibrin-strands build a mesh in which blood components can be caught, which results in forming of a blood clot [5].

Traumatized vascular wall including endothelial cells and subendothelial collagen fibers, platelets and adhered blood proteins release activating substances, which lead to the formation of prothrombin activator, which induces all other clotting steps.

Prothrombin activator can be formed by two pathways: (1) *the extrinsic pathway* and (2) *the intrinsic pathway*.

The intrinsic pathway is slower and all essential factors required to activate it, can be found at any time in physiological blood and that is why it is called 'intrinsic'. The extrinsic pathway is activated by factors, which are not found in blood, e.g. tissue factor (TF). It is activated by tissue injury with cell destruction, while the intrinsic pathway is activated by endothelium defects. These two pathways affect each other and converge at the level of Factor X. From this level on they have a final common pathway, which leads to activation of thrombin and formation of fibrin [2,4,5]. A summary of the coagulation cascade, with both pathways, their activating factors and co- factors and their final common pathway is illustrated in figure 1.

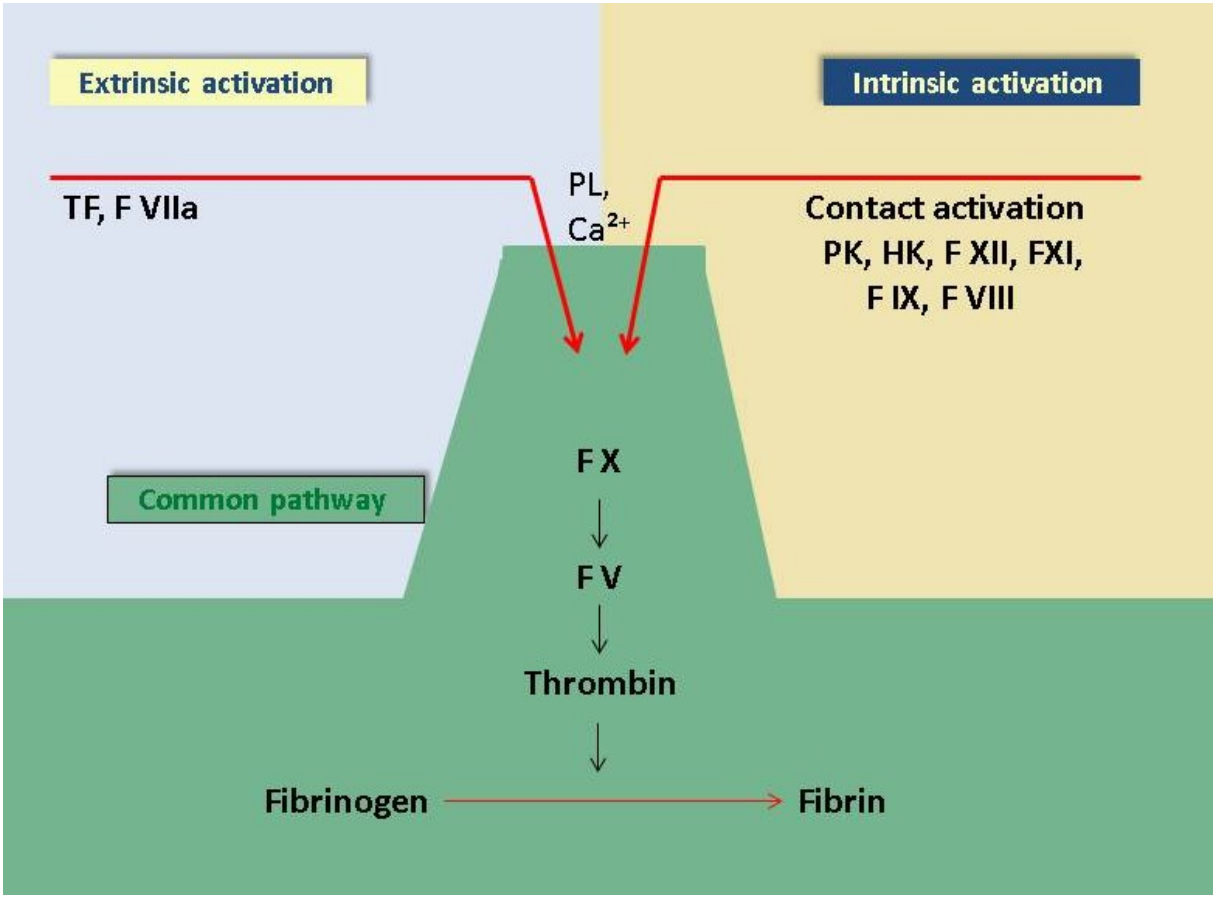

Figure 1: The coagulation cascade [8]

Extrinsic Pathway

"Causal agent" for the formation of prothrombin activator in this pathway is expression of TF from the traumatized vascular wall or traumatized extravascular tissue. This factor is also known as tissue thromboplastin. This process occurs in 3 steps. During the first step TF is released. The second step is characterized by the activation of factor VII. The activation of factor X also, ensues as a result of this step. The complex called prothrombin activator is formed at the third step [2,5,8]. An illustration of this pathway can be seen in figure 2.

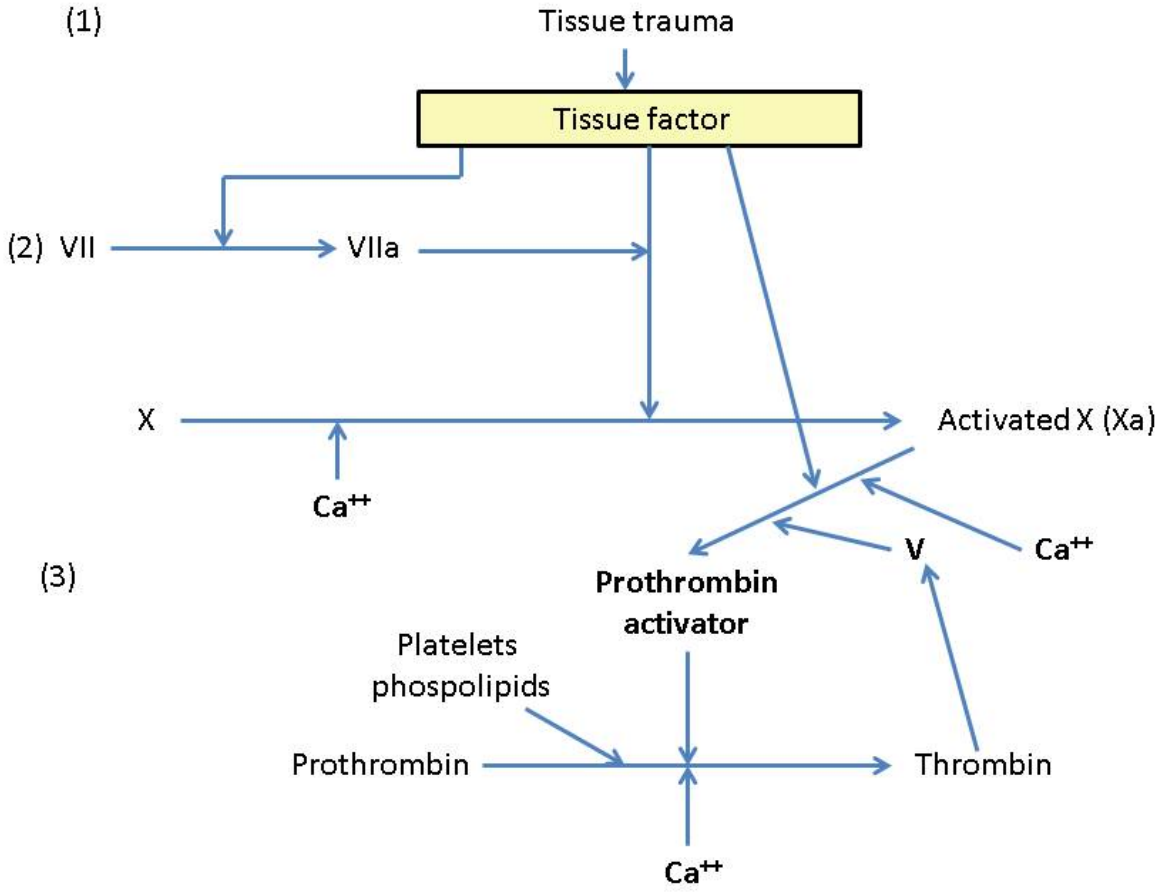

Figure 2: Extrinsic pathway for initiating blood clotting [2]

Intrinsic Pathway

All the components necessary for this pathway are present in circulating blood. They just have to be activated [1,5]. In vivo they can be activated when blood comes into contact with subendothelial collagen fibers while in vitro, they are activated when blood contacts with glass. Both causal factors are produced as a result of tissue damage. [2,3,8].

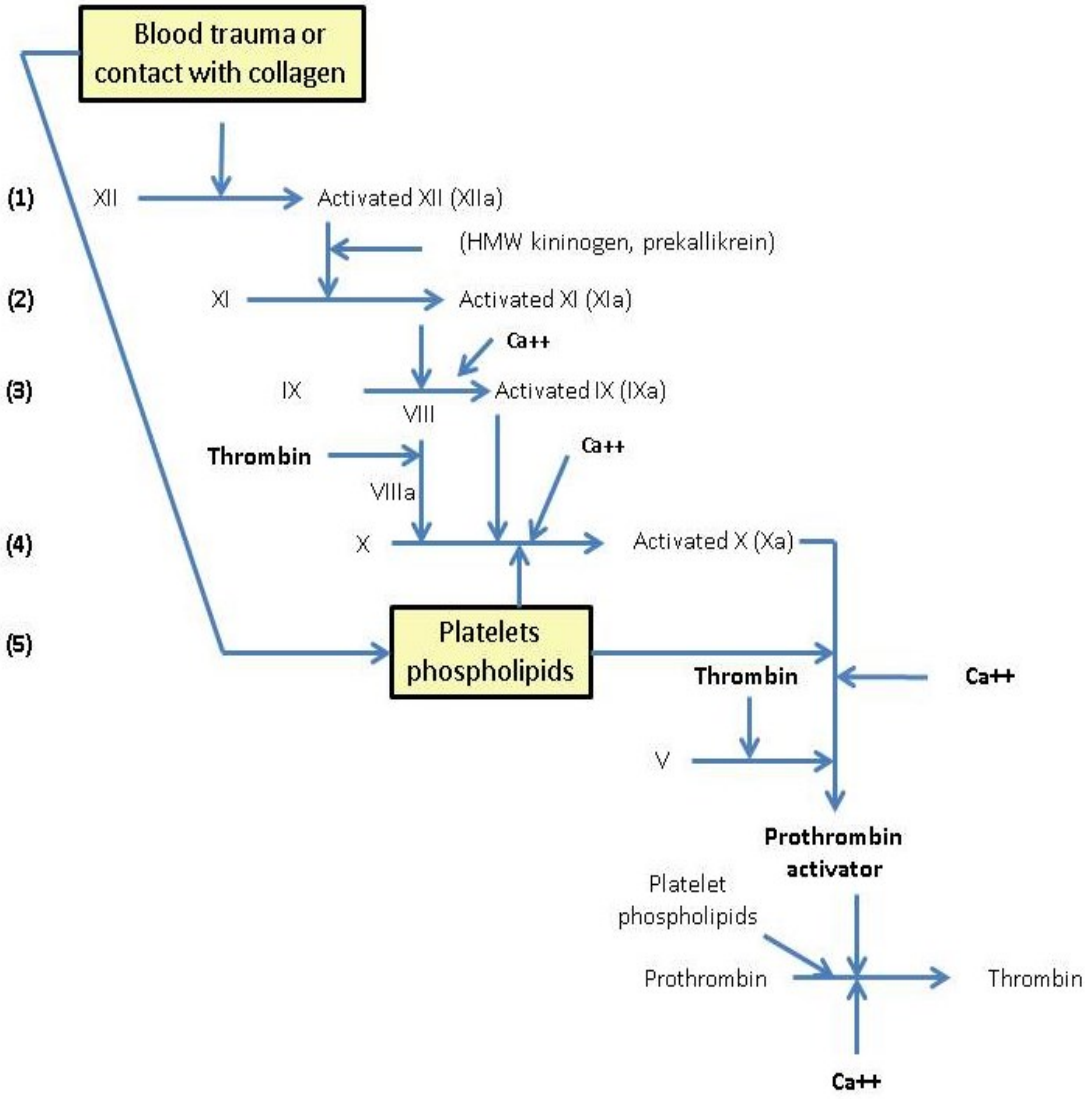

Figure 3: Intrinsic pathway for initiating blood clotting [2]

Figure 3 shows the steps of the intrinsic pathway. Contact of blood with negatively charged surfaces leads to the initial reaction of this pathway: (1) the conversion of inactive factor XII to active factor XII (XIIa) and also alters the platelets. The platelets are damaged and release phospholipid. (2) Factor XI is activated. (3) Activated factor XI

activates factor IX. (4) The complex of activated factor IX and activated factor VIII initiates the activation of factor X. (5) The complex called prothrombin activator is formed.

1.1.3. Coagulation Inhibitors

Blood and tissue contain more than 50 important substances, which cause or affect blood coagulation. Some of them, known as procoagulants, promote coagulation. Others, also known as anticoagulants, inhibit blood coagulation. Dominance of anticoagulants prevents blood coagulation in healthy blood vessels. After the injured area was mended by a clot, activated blood coagulation has to be stopped [2,8]. There are two inhibitor systems in plasma, which keep coagulation under control: (1) protease inhibitors and (2) the protein C pathway.

1) Protease inhibitors inhibit activated coagulation factors. This group includes, among other inhibitors, the tissue factor pathway inhibitor (TFPI) and antithrombin. The TFPI inhibits the activated Factor X (F Xa) and the F VIIa/ TF-complex. Antithrombin inhibits the Factors IIa, Xa, and IXa.

2) The protein C pathway leads to the inactivation of activated cofactors, particularly the co-factors F Va and F VIIIa. The protein S as a co-factor accelerates this inhibiting reaction [6,8].

1.1.4. Physiological Changes of Hemostasis during Pregnancy

Pregnancy is known as a state of hypercoagulation [15,16,19]. As a part of the process of female body adaptation to pregnancy, hypercoagulation aims to hinder bleedings from the placenta during its development [18] as well as to prevent fatal hemorrhage during delivery and puerperium [9]. Nevertheless, pregnant women run an increased risk for thrombosis and venous thromboembolism (VTE) [9,10], which is one of the most common mortality reasons during pregnancy in developed countries [19].

Hypercoagulation during pregnancy stems from plasma changes, specifically, from procoagulant factors, natural anticoagulant factors and markers of fibrinolysis [11]. It reaches its peak around the delivery date, and during the post-partum period [10]. Blood changes caused by pregnancy, return to non-pregnant women's values, approximately 4 weeks after delivery [13]. An overview of plasma changes in pregnant women is shown in table 1.

During pregnancy, the levels of coagulation factors II, VII, VIII, X, and fibrin, are increased. Fibrinolytic activity and free protein S levels are decreased [17,19]. It is considered, that during pregnancy, the efficacy of the activated protein C (APC) system is also affected. This causes an acquired APC resistance, which appears in up to 60% of pregnancies [16].

Most hemostatic changes during pregnancy are a result of increased estrogen level [9,12].

Compared to non- pregnant women, pregnant women have a 4 to 5 times higher risk for VTE [20,21]. In contrast, women who are using oral contraceptive (OC) or hormone replacement therapy (HRT) are running a 2- to 6- fold or 2- to 4- fold increased thrombosis risk [20].

Table 1: Major changes in haemostasis factors during pregnancy in relation to non-pregnant state. [10]

Parameter	Change*
Platelet count	↓
Fibrinogen, von- Willebrand factor	↑
Factors VII, VIII, IX, X, XII	↑
Factor XI	=/↓
Factors V, XIII	↑/↓
Antithrombin, protein C	=
Protein S	↓
Tissue plasminogen activator	↓
Plasminogen activator inhibitor- I, thrombin-activatible fibrinolysis inhibitor	↑
Prothrombin fragment 1 + 2, thrombin-antithrombin complex, D-dimer, fibrinopeptide A	↑

*The symbols represent: ↑, increase; ↓, decrease; =, no change

1.2 Thrombophilia

Thrombophilia represents a disorder of hemostasis that predisposes a person to develop thrombotic events [19,24]. Thrombophilia can be inherited, acquired or mixed, which means that environmental influences, such as diet or other lifestyle factors, interact with the genetic background of the individual. [22,24,25].

Most patients with inherited thrombophilia can remain free of symptoms for a long period of time and first manifestation of thrombosis occurs at later age [24,25]. Recurrent VTE, thrombosis of an unusual location (cerebral sinuses, mesenteric, portal), family history of thrombosis, VTE at young age (< 45 years) are conditions that make one person suspicious for thrombophilia [24,25].

Pregnancy failure (recurrent miscarriage or late abortion), as well as hypertensive pregnancy complications (preeclampsia and HELLP- (elevated liver enzymes, and low platelets) Syndrome) can be associated with thrombophilic disorders [23,24,26].

Conditions, in which it should be think about thrombophilia are listed in table 2.

Table 2: Most Common Features of Thrombophilia

• **Reccurent venous thromboembolism**
• **Thrombosis at an unusual location (such as cerebral sinuses, mesenteric, portal)**
• **Venous thromboembolism at a young age**
• **Family history of venous thromboembolism**
• **Pregnancy failure (such as sporadic and recurrent miscarriage, late abortion)**
• **Hypertensive pregnancy complications (such as preeclampsia and HELLP- (elevated liver enzymes, and low platelets) Syndrome)**

1.2.1 Inherited Thrombophilia

In most inherited thrombophilias, the mechanisms of thrombosis are (1) reduced neutralization of thrombin or (2) failure of thrombin generation control. Therefore, inherited thrombophilias are characterized either by decreased neutralization of thrombin or increased generation of thrombin. These mechanisms lead to dysfunctions in a system of natural anticoagulants, which regulates fluidity of blood. Antithrombin is a natural anticoagulant responsible for neutralization of thrombin, factor Xa, factor IXa and factor XIa. Another natural anticoagulant, which controls thrombin, is protein C.

Deep-vein thrombosis, pulmonary embolism, or both of them, are common clinical manifestations of inherited thrombophilia. Rare clinical events are superficial venous thrombosis and thrombosis of cerebral, visceral, and axillary veins. Triggers for venous thrombosis are, in more than 50%: any kind of surgery, immobilization, pregnancy and treatment with OC or HRT [26]. Increased risk of arterial thrombosis is not significantly associated with inherited thrombophilia [24]. Table 3 provides an overview of all possible factors for inherited thrombophilia sorted by their frequency.

Table 3: Inherited factors of venous thrombosis; modified according to [26]

Inherited Thrombophilia	**Common**	• G1691A mutation in the factor V gene (factor V Leiden) • G20210A mutation in the prothrombin (factor II) gene • Homozygous C677T mutation in the methylenetetrahydrofolate reductase gene
	Rare	• Antithrombin deficiency • Protein C deficiency • Protein S deficiency
	Very rare	• Dysfibrinogenemia • Homozygous homocystinuria
	Probably inherited	• Increased levels of factor VIII, factor IX, factor XI, or fibrinogen*

*Levels of factor VIII and fibrinogen may also increase as part of the acute-phase response.

Antithrombin deficiency and dysfibrinogenemia were the first described modes of inherited thrombophilia. Thereafter, protein C and S deficiencies were identified as reasons of inherited thrombophilia. In the beginning, due to the rarity of these deficiencies, results of patients with idiopathic venous thrombosis who were tested for inherited thrombophilia were disappointing. Only 5% to 20% of patients were diagnosed positive for one of these factors. Detection of resistance to activated protein C in 1993, changed this situation. In 1996, another very common reason of thrombophilia called prothrombin 20210 mutation was discovered [26].

Most prevalent pathogenic risk factors of thrombosis are resistance to activated protein C, due to a single point-mutation in the factor V gene and the G20210 prothrombin mutation

[24,26,27,33]. The hereditary form of APC resistance represents the mutation in factor V gene, which is called 'factor V Leiden' [8].

Prevalence of factor V Leiden in Europe varies between 2 to 7% and prevalence of the prothrombin 20210 mutation varies between 1 to 3 % [24]. These two mutations are extremely rare in Asia and Africa [24,26]. The worldwide prevalence of these two most common reasons for inherited thrombophilia is shown in figure 4.

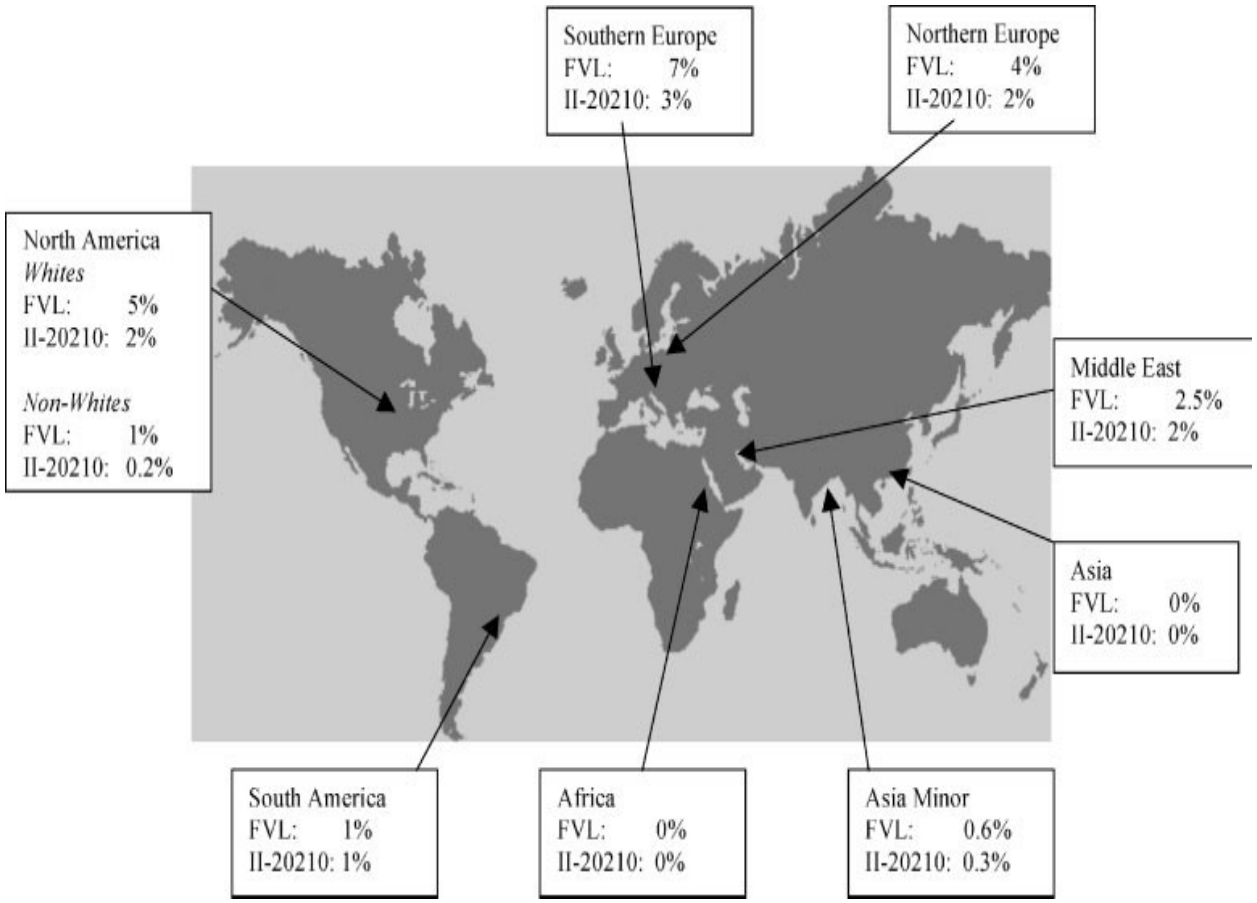

Figure 4: Geographic distribution of the prevalence of the two most common forms of the thrombophilia (factor V Leiden [FVL] and prothrombin 20210 [II-20210] mutation) [24]

1.2.1.1 Activated Protein C Resistance

Protein C pathway has an important role in the control of activated coagulation [8]. Defects in the protein C pathway, result in "Resistance to Activated Protein C" (APCR) [8,21], which is the most common congenital disorder associated with thrombophilia [8].

Protein C is a vitamin K dependent molecule, which inactivates factors Va and VIIIa. Inactivation of factors Va and VIIIa occurs only when protein C is activated and when

protein S, another vitamin K dependent plasma protein and factor V are present as co-factors.

In the first step of protein C activation, thrombomodulin has to be activated by thrombin. In addition, plasma protein C binds to thrombomodulin to build activated protein C [8,32]. The term APCR describes a malfunction of the protein C pathway, characterized by a reduced response of plasma to APC. Reasons for this condition can be inherited (Factor V Leiden) or acquired [8].

Elevated FVIII levels, prothrombin mutation G20210A, deficiencies of antithrombin, protein C and protein S are inherited reasons, OC use, pregnancy, HRT, cancer and antiphospholipid antibodies are acquired risk factors associated with plasmatic APC-Resistance [8,34].

1.2.1.2 Factor V Leiden

Factor V Leiden is a major reason of hereditary APCR [8,35]. Then again, APCR due to factor V Leiden mutation is the most common inherited reason for inherited thrombophilia [26,27]. Prevalence of factor V Leiden (FVL)- Mutation varies [35]. Table 4 offers an insight into worldwide prevalence of factor V Leiden. The highest prevalence of FVL-Mutation can be found at the Mediterranean region [36]. Figure 5 shows the prevalence of factor V Leiden in the 20 countries of the Mediterranean region.

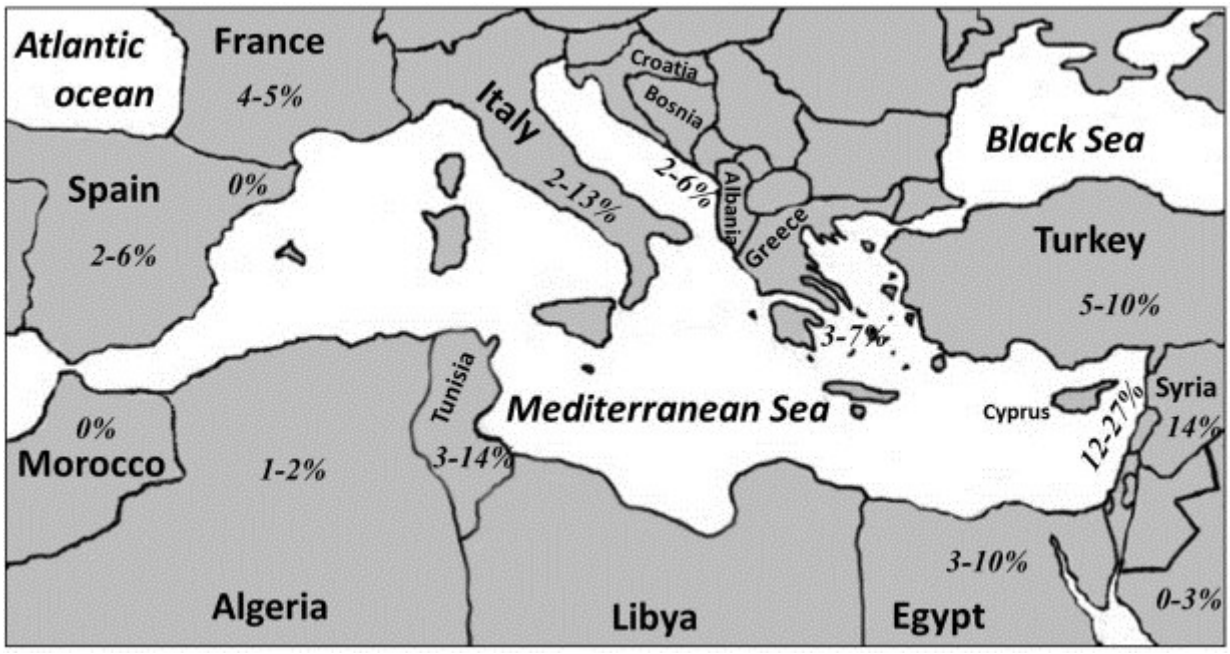

Figure 5: Map of the Mediterranean Sea and its countries showing the prevalence of FVL in healthy populations living there [36]

With a prevalence of 2 to 10% in the Caucasian origin, factor V Leiden mutation is ten times higher than other genetic condition associated with VTE [31].

A point mutation of the factor V Leiden gene results in substitution of guanine to adenine at nucleotide 1691 (1691 G→ A). Due to replacement of arginine by glutamine at position 506 (Arg506Glu) of the factor V protein, the emerging protein cannot be appropriately inactivated by APC (it is called factor V Leiden) [26,28]. On the end, production of thrombin is increased [8]. An illustration of FVL-Mutation can be seen in figure 6.

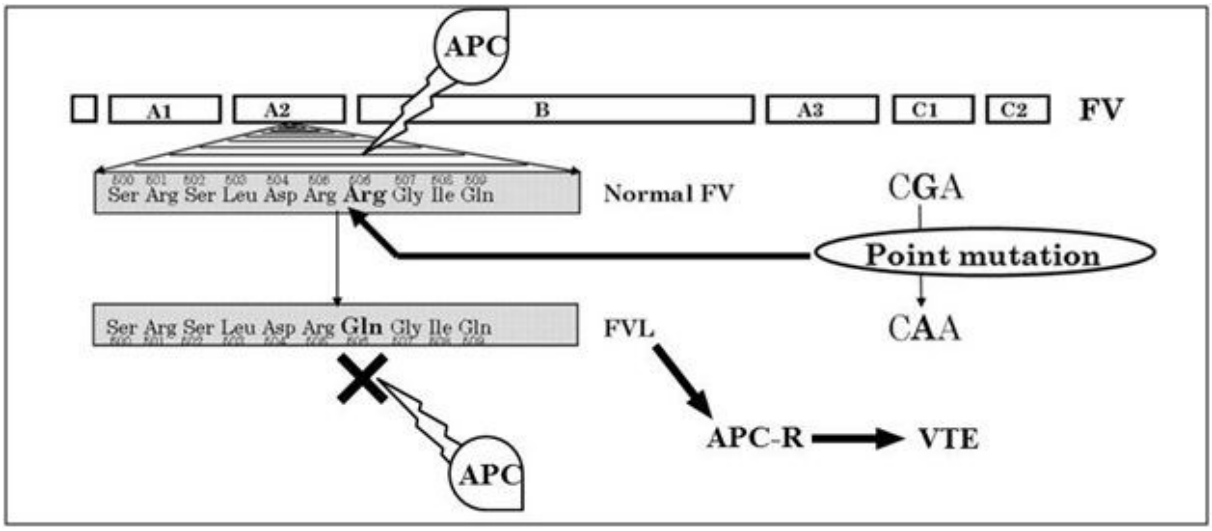

Figure 6: Factor V Leiden-Mutation [36]

This genetic condition is inherited autosomal dominant [29]. Heterozygous individuals have a five-fold increased risk and homozygous individuals a 50- to 100-fold increased risk for thromboembolic events [31]. Around 50 % of factor V molecules, in heterozygous Factor V Leiden patients, do not show this mutation. That is why they develop thromboembolic events less frequently compered to homozygous individuals [8].

Table 4: Worldwide prevalence of Factor V Leiden modified according to [35]

Population	Prevalence (%)[a,b]
European whites	3-15
Spain	3.3
France	3.8
Germany	4
Iceland	5.2
United Kingdom	8.8
Greece	15
Sweden	11
Africa	Absent
Southeast Asia	Absent
Asia minor	1.2
Australia (indigenous)	Absent
Japan	Absent
Jordanian Arabs	12.2
Lebanon	14
Western Iran	2.97
Canada	5.3
United States	
Whites	5.2
Hispanic Americans	2.2
African Americans	1.2
Asian Americans	0.45
Native Americans	1.25

[a]Healthy individuals with no history of venous thromboembolism.
[b]Includes heterozygous and homozygous individuals.

1.2.1.3 Prothrombin gene mutation

Prothrombin gene mutation is the second most common genetic abnormality for thrombophilia [37]. This mutation is characterized by a guanine to adenine transition in position 20210 in the 3' untranslated region of prothrombin gene (G20210A) [25,32,37].

Prothrombin gene mutation has an autosomal dominant inheritance pattern. Homozygous individuals have an up to 70%, heterozygous a 30% higher plasma-prothrombin-level than

healthy individuals [37]. Prevalence of G20210A mutation is 2% in northern Europe and up to 6,5 % in southern Europe [25].

1.2.1.4 Antithrombin Deficiency:

As primary inhibitor of thrombin and of the other clotting factors, antithrombin (AT) plays a major role in fibrin formation and due to that, against blood coagulation [25,38]. AT has also an important anti-inflammatory effect because of its interaction with endothelium [38]. Table 5 gives an overview about types of AT- Deficiency.

Table 5: Types of AT- Deficiency modified according to [25,32,37,38]

Types of Antithrombin Deficiency		
Type of defect	Defect?	Prevalence in general population
Type I - Quantitative	**Synthesis of qualitatively normal protein is reduced**	0,0002-0,002%
Type II - Qualitative	**Quantitatively normal synthesis of qualitatively abnormal protein**	
Subtypes	Defect of	
IIa	Thrombin-binding Domain	
IIb	Heparin-binding Domain	
IIc	Pleotropic	

1.2.1.5 Protein C Deficiency

Protein C (PC) is vitamin K dependent. It is synthesized in the liver and circulates in plasma as an inactive precursor. In order to manifest its anticoagulant activity, PC has to be activated by thrombin. APC inactivates the cofactors FVa and FVIIIa. In order to do so, APC needs: protein S, FV, calcium ions and phospholipids [39].

The first patient with protein C deficiency was described in 1981 [37, 39]. Protein C deficiency is inherited in an autosomal dominant pattern [32, 37]. This disorder can be subdivided into two types, quantitative deficiency (type I) and qualitative deficiency (type II). Type I deficiency is characterized by reduced production of protein C and its short activity. Production of dysfunctional variants of protein C characterizes type II protein C deficiency [37, 39].

Prevalence of this defect among general population is approximately 0.3% [25]. This disorder is rare, but when other thrombotic risk factors are present, such as factor V Leiden, protein C deficiency becomes a clinical significant disorder [39].

1.2.1.6 Protein S Deficiency

Protein S (PS) is also a vitamin K dependent protein, which circulates in plasma in a free form and as partly bound to the C4b binding protein. The percentage ratio of these two circulating PS forms is 40% to 60% [25,40]. Both forms possess APC cofactor activity and are involved in inactivation of the activated cofactors F Va and F VIIIa of the coagulation cascade. Therefore, protein S plays also an important part in controlling thrombin generation and fibrinolysis [40].

Concentrations of PS are reduced during pregnancy, in women using combined OC or in case of inflammation. This leads to an acquired protein C deficiency [8,25,40].

PS deficiency is inherited autosomal dominant [40]. Based on following parameters: total PS antigen concentration, free PS concentration, and PS functional activity, PS deficiency is subdivided into three types [32]. Type I (the most common one) is characterized by low activity of total and free PS [32,37]. Type II is associated with low PS activity [37]. Type III has a low activity and free PS [32,37].

Homozygous and compound heterozygous PS deficiency is extremely rare and lethal without treatment [37,40]. Heterozygous PS deficiency is observed more often [37].

Prevalence of this disorder among Caucasians is estimated about 0.3 to 0.13 % [32,37].

1.2.1.7 Homocysteinemia/ MTHFR-Mutation

Hyperhomocysteinemia is a disorder characterized by elevated plasma concentrations of homocysteine [8,25]. That may be caused by genetic or acquired defects [25,26]. However, this condition is associated with an increased risk of venous thrombosis, as well as with an increased risk of arterial thrombosis [25,26,32]. MTHFR mutations are also associated with a neural tube defect (spina bifida) and with other pregnancy complications (including preeclampsia, placental abruption, recurrent pregnancy loss, and intrauterine growth restriction (IUGR)) [41].

Acquired reasons resulting in hyperhomocysteinemia may be folic acid deficiency, vitamin B_{12} deficiency, vitamin B_6 deficiency, renal failure, hyperthyroidism and smoking [26].

Mild hyperhomocysteinemia is present in 5 to 7 % of general population [32].

1.2.2 Acquired Thrombophilia

Conditions for an acquired (not inherited) thrombophilia can be various (Table 6).

Table 6: Inherited causes of venous thrombosis; modified according to [26]

Acquired Thrombophilia	• Surgery and trauma • Prolonged immobilization • Older age • Cancer • Myeloproliferative disorders • Previous thrombosis • Pregnancy and puerperium • Use of contraceptives or hormon-replacement therapy • Resistance to activated protein C that is not due to alterations in the factor V gene • Antiphospholipid antibodies • Mild-to-moderete hyperhomocysteinemia

1.2.2.1 Acquired Activated Protein C Resistance

The term acquired APCR describes the condition in which a person develops resistance to APC in absence of the FVL mutation [16,37]. Hence, this condition is also termed as non-factor V Leiden APCR [30,37].

Acquired APCR is associated with several changes of hemostasis, for example: high concentration of factor VIII, PC deficiency, PS deficiency or increased AT levels [20,25,37].

Pregnancy is a state of elevated endogenous hormones [20] and altered clotting factors [37]. Pregnant women have an increased risk of developing acquired APCR [20,30,37].

Application of exogenous estrogen, in form of OC or HRT, is associated with alterations in plasma concentrations of almost all coagulation factors and anticoagulant proteins. Individuals who are using one of these treatments have an increased risk to develop a non-factor V Leiden APCR [20,30,37].

Antiphosholipid antibodies, such as lupus anticoagulants and anticardiolipin antibodies may account for a reduced response to APC [30,37].

1.2.2.2 Acquired Autoimmune Thrombophilia

1.2.2.2.1 Antiphospholipid Syndrome

Antiphospholipid Syndrome (APS) represents a systematic autoimmune vascular disease linked to recurrent thrombosis and pregnancy morbidity [10,37,42]. It is the most common and most important acquired thrombophilic disorder [25]. The presence of antiphospholipid antibodies (aPL) in serum provokes this disorder [42]. aPL includes the lupus anticoagulant (LA), anticardiolipin antibody (anti-CL) and anti-beta 2 glycoprotein 1 (anti-β2GPI) [23,37]. Therefore, aPLs belong to a heterogeneous group of antibodies, but all of them have an influence on the physiological mechanism of coagulation and fibrinolysis [37,42].

APS can be divided into 1) primary antiphospholipid syndrome, without association with systemic disorder, and 2) secondary antiphospholipid syndrome, which occurs in association with connective tissue disorders called systemic lupus erythematosus [10].

The diagnosis of the APS is based on the Sapporo classifications criteria, revised in 2006, in Sidney, Australia. These criteria are also called the Sidney criteria [37,42,43]. The following Table (Table 7) offers an overview of the classification criteria for the APS.

Table 7: Revised classification criteria for the antiphospholipid syndrome; Modified according to [37,42,43]

Clinical criteria	*Vascular thrombosis* One or more clinical episodes of arterial, venous, or small vessel thrombosis, in any tissue or organ.
	Pregnancy morbidity a. One or more premature birth of normal neonate before 34 weeks of gestation b. One or more unexplained death of a normal foetus at or beyond 10 weeks of gestation c. Three or more unexplained spontaneous abortions before 10 weeks of gestation
Laboratory criteria	1. Lupus anticoagulant present in plasma on two or more occasions at least 12 weeks apart, detected according to the guidelines of the International Society on Thrombosis and Haemostasis 2. Anticardiolipin antibody of IgG and/or IgM isotype in serum or plasma, present in medium or high titer on two or more occasions at least 12 weeks apart, measured by a standardized ELISA 3. Anti-b2 glycoprotein I antibody of IgG and/or IgM isotype in serum or plasma, present on two occasions at least 12 weeks apart, measured by a standardized ELISA

According to the revised Sapporo criteria, APS is diagnosed if at least one of the clinical criteria and at least one of the laboratory criteria in table 7 occur [23,37,42,43].

1.2.2.2.2. Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by inflammatory affection of the skin, joints, serous membranes, kidneys and central nervous system.

This disorder can affect both genders at any age, but SLE primarily occurs in women of a childbearing age. During pregnancy it increases the risk of adverse maternal and fetal outcomes [44,45,46].

The American College of Rheumatology published in 1982 the first form of classification criteria for SLE. In 1997 the American College of Rheumatology updated these classification criteria. The diagnosis of SLE is based on the presence of at least four from eleven in Table 8 listed criteria.

Table 8: Summary of the American College of Rheumatology Classification Criteria for Systemic Lupus Erythematosus modified according to [46]

Criterion
1. Malar rash
2. Discoid rash
3. Photosensitivity
4. Oral ulcers
5. Arthritis
6. Serositis
7. Renal disorders
8. Neurological disorders
9. Hematologic disorders
10. Immunologic disorders
11. Positive antinuclear antibodies

The interaction of both, genetic and environmental factors contributes the development of SLE, but its cause remains mostly unknown [46].

About 40 to 60% of SLE patients have aPL antibodies, such as LA and aCL antibodies. The aPL antibodies enhance the risk of organ damage in SLE patients. Compared to aPL-patients without other systemic disease, thrombotic complications appear more frequently in patients with SLE [37,46].

1.3. Factor V Leiden and Pregnancy

Pregnancy itself is known as a physiological hypercoagulability state [15,19,47]. On the one hand, this hypercoagulability condition safes pregnant women from bleeding during placentation and third stage of labor, but, on the other hand it increases maternal risk for venous thromboembolism during pregnancy [18]. Factor V Leiden is an additional risk for venous thromboembolism [47].

Pregnancy- related venous thromboembolism is not the only complication that can occur at women with thrombophilia during pregnancy. Due to placental vascular thrombosis and abnormal placentation, risk of placenta mediated pregnancy complications is also given. Common placental mediated pregnancy complications are preeclampsia, small for gestational age infants, placenta abruption, miscarriage, and stillbirth. Genetic thrombophilias increase the risk for this group of complications [48].

1.3.1. Pregnancy- related Venous Thromboembolism

Compared to not-pregnant women, pregnant women have approximately three to twenty times increased risk for venous thromboembolism. Based on the fact that 12-15% of maternal deaths in pregnancy are caused by venous thromboembolism, thromboembolism is a major cause of morbidity and mortality among pregnant women.

Additional factors that increase the risk for VTE during pregnancy are: mode of delivery (after Caesarean section women have a 3-5 times higher risk of thrombosis than after vaginal delivery), age > 35 years, multiparity, obesity, immobilization, and thrombophilia of any origin [25,47,49]. Women carrying the FVL-Mutation when compared to pregnant women without thrombophilia have an increased risk for pregnancy- related venous thromboembolism [50,51].

1.3.2. Factor V Leiden and Hypertensive disorders in Pregnancy

Preeclampsia is diagnosed if a pregnant woman develops both, gestational hypertension and proteinuria after the 20th week of gestation. Gestational hypertension, also called pregnancy-induced hypertension (PIH) is a condition of high blood pressure (blood pressure ≥140/90 mm Hg). Proteinuria describes the state in which urine contains an abnormal amount of serum protein (≥ 300mg/24h) [52-54].

There are two types of Preeclampsia: 1) mild preeclampsia and 2) severe preeclampsia. Mild preeclampsia is characterized by blood pressure of up to 160/110 mm Hg and

proteinuria of up to 5g/24h. Severe pre-eclampsia is diagnosed if an additional criterion exists [52-54]:

- Restriction of renal function (creatinine ≥0,9 g/L or oliguria, urinary output <30 mL/h during 3h)
- Liver involvement (increase in transaminases, epigastric and right upper quadrant pain)
- Hematological disorders (thrombocytopenia, hemolysis)
- Oxygen saturation < 90%, pulmonary edema or cyanosis
- Neurological symptoms (headache or visual disturbances)
- Intrauterine growth restriction (IUGR)
- Systolic blood pressure ≥160 mm Hg and/or diastolic blood pressure ≥110 mm Hg
- Proteinuria ≥ 5 g/24h

Chronic hypertension is defined as high blood pressure (systolic blood pressure >140 mm Hg and/or diastolic blood pressure >90 mm Hg), which is diagnosed before the 20th week of pregnancy and still exists 12 weeks after delivery [52-54].

Eclampsia is characterized by occurrence of an eclamptic seizure. Seizure can appear before delivery (53%), during delivery (19%), as well as in the postpartum period (28%). Eclampsia can be associated with severe preeclampsia (50%) or appear in patients without hypertension or proteinuria (14-34%) [52-54].

Robertson et al described a significant association between preeclampsia and FVL [51].

The acronym HELLP in pregnancy disorder called HELLP-Syndrome stands for:

- ‘H’- hemolysis,
- ‘EL’- elevated liver enzymes,
- ‘LP’- low platelets.

HELLP Syndrome can occur related to preeclampsia as well as in pregnancies without the diagnosis of preeclampsia. 20% of women with diagnosed HELLP-Syndrome are normotensive, 5-15% of them do not have proteinuria, and 15 % have either high blood pressure or proteinuria [52-54].

The study by Schlembach et al. showed a twofold elevated prevalence of maternal FVL in HELLP patients compared to the control group, which consisted of women without HELLP-Syndrome [55].

About a significant association between FVL and preeclampsia/HELLP-Syndrome reported the study by Benedetto et al. [56].

Fig. 7 gives an overview of possible complications associated with hypertension during pregnancy.

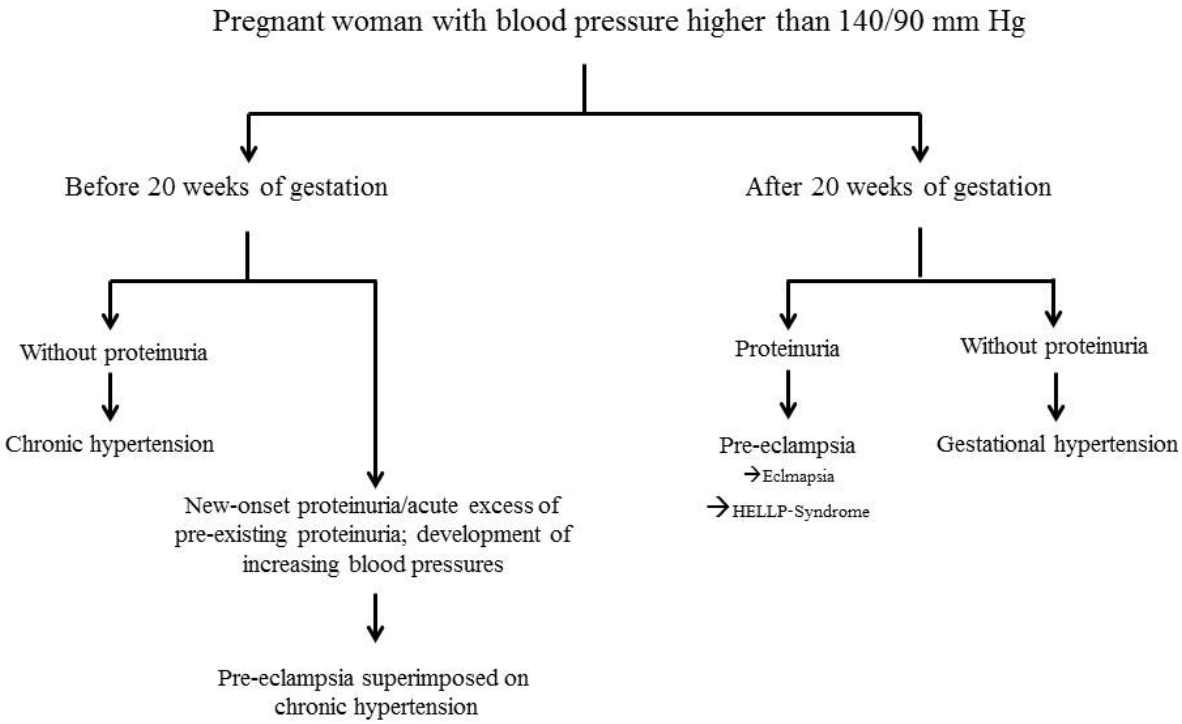

Figure 7: An algorithm for differentiation among hypertensive disorders in pregnant women. Modified according [52]

1.3.3. Factor V Leiden and Placental Abruption

Abruption placentae is a complication, characterized by ante partum retroplacentar, marginal, or preplacentar hemorrhage, that can be confirmed either by imaging studies or inspection of the placenta [22].

Rodger et al described placenta abruption in FVL positive women with a prevalence of 1%, as a rare pregnancy complication [48]. Its risk is increased in women with FVL compared to women without this mutation [22,51].

1.3.4. Factor V Leiden and Intrauterine Growth Restriction

Suggested by the American College of Obstetricians and Gynecologists, the term intrauterine growth restriction (IUGR) describes any fetus that fails to reach his potential growth. Small for gestational age (SGA) is defined as a weight below the 10th percentile (10%) for a given gestational age. These two terms are not synonyms, and the term IUGR should be only used for the fetus, whereas the term SGA should be used just for newborns [57].

A Systematic review by Robertson et al. and a meta- analysis by Facco et al. reported about increased IUGR risk in pregnant women with FVL [51,58].

1.3.5. Factor V Leiden and Miscarriage/Abortion

Abortion or miscarriage is the spontaneous loss of a fetus before the 20th week of pregnancy or when the fetus weighs less than 500g. Based on the trimester in which abortion occurs, abortions can be divided in to two groups. Those abortions that occur in first trimester belong to the early abortion group. Late abortion group includes abortions that occur in second trimester. Habitual abortion or recurrent pregnancy loss is defined as three or more abortions with the same partner [56].

A systemic review by Robertson et al. showed significant association for pregnancy loss among women with FVL [51].

1.3.6. Factor V Leiden and Intrauterine Fetal Death

Intrauterine fetal death (IUFD) is defined as a fetal loss at or after 24th week of pregnancy and when the fetus weighs more than 500 g [47,56].

The studies by Grandone et al. and Middeldorp S. reported about significant association between FVL and IUFD [47,23].

1.4. Screening for Thrombophilia in Pregnancy

During pregnancy women undergo homeostatic changes that increase their risk for VTE and other obstetric complications. This risk is further increased due to thrombophilia. How strong the association between thrombophilias and pregnancy complications is, depends on the type of obstetric complications (e.g. recurrent pregnancy loss, IUFD, IUGR, abruption placentae, preeclampsia and HELLP-Syndrome) and on the type of thrombophilia.

The meta-analyses of heterogeneous case-control studies showed an increased prevalence of FVL in women with pregnancy complications [59].

The absolute risk of pregnancy-related VTE and obstetric complications, obtained from cohort studies and systemic reviews is estimated to be low. Among women with diagnosed FVL-Mutation, the absolute risk of VTE is about 0.8% and the absolute risk of obstetric complications is even lower [59].

Optimal moment to conduct the test for thrombophilias is before pregnancy [59].

Suggested by Italian Society for Haemostasis and Thrombosis (SISET), indications for thrombophilia screening in pregnancy are summarized in table 9.

Table 9: Indications for thrombophilia screening among pregnant women; modified according to [59]

Asymptomatic women:
• Family history of inherited thrombophilia • Family history of venous thromboembolism • Family history of obstetric complications*
Symptomatic women:
• Women with history of venous thromboembolism • Women with history of previous obstetric complications*

* recurrent pregnancy loss, intrauterine fetal death, preeclampsia, HELLP-Syndrome, placental abruption

1.5. Factor V Leiden and Anticoagulation Therapy/Prophylaxis during Pregnancy

Factor V Leiden is not proven to be a reason for placenta-mediated pregnancy complications, but FVL is one of many factors that increase the prevalence of these complications [60].

Due to micro-and/or macro-vascular thrombosis and abnormal placentation, thrombophilias affect the slow low-pressure circulation in the placenta. This can lead to development of placenta-mediated pregnancy complications [48].

Prevention of blood clotting due to antithrombotic drugs can be useful as prophylaxis of pregnancy-related VTE and placenta-mediated pregnancy complications [59].

1.5.1. Anticoagulation drugs

1.5.1.1. Heparins

Unfractionated heparin (UFH) and low-molecular-weight heparin (LMWH) are two types of heparins that are used as anticoagulants during pregnancy. Beside their anticoagulant effects, heparins have further significant effects. LMWH stimulates expression of heparin-binging epidermal growth factor-like growth factor (HB-EGF), which positively impacts the process of placentation during first trimester. In the first trimester, there are divers signals that induce apoptosis of cytothrophoblast cells. This can be inhibited by UFH [60].

Because of their high molecular weight, heparins do not penetrate the placental barrier. Based on that fact, heparins do not increase the incidence of fetal hemorrhage or teratogenity, what is confirmed by several studies. UFH and LMWH are not secreted in breast-milk and nursing mothers can safely use them as anticoagulant drugs [60].

Maternal complications that can occur for pregnant women undergoing a treatment with LMWH or UFH are: heparin-induced thrombocytopenia (HIT), major antepartum bleeding, osteoporosis (by long-term treatments), bruising, local allergic reaction, and pain at injection sites for heparin-related compounds. The incidence of these complications is generally lower at pregnant women receiving LMWH compared to pregnant women receiving UFH. For this reason, the study by Bates et al. recommends LMWH administration for pregnant women, instead of UFH administration [61].

1.5.1.2. Aspirin

As an antiplatelet agent, aspirin is also used as an antithrombotic drug. Due to its inhibition of platelet aggregation, Aspirin blocks the formation of blood clot [60].

Aspirin does penetrate the placental barrier, and several animal studies showed increased risk for congenital anomalies. Examination of safety of aspirin use in pregnancy, conducted due to several systemic reviews, showed that aspirin use is associated with occurrence of gastroschisis with an Odds ratio (OR) of 2.37 (95% CI 1.44-3.88). For safety treatment, Aspirin should be dosed low. The Daily dose of aspirin should be about 80-150 mg [60,61].

Low-dose aspirin in combination with LMWH can be given to pregnant women with either mechanical heart valves or APS [60].

1.5.2. Recommendations for Prophylaxis in Women with FVL

In current literature, there is much debate about antithrombotic treatment of pregnant women because of its potential fetal and maternal complications. However, treatment is suggested because the majority of the studies showed significantly better pregnancy outcome in treated pregnancies compared with pregnancy outcomes in untreated pregnancies [47].

Thromboprophylaxis in pregnant women differs from the thromboprophylaxis in non-pregnant women. Dosages and monitoring guidelines used to treat not pregnant women cannot be easily applied as antithrombotic treatment in pregnant women. The treatment has to be adapted.

As ‘‘low-risk’’ thrombophilia, FVL should be treated with UFH or LMHW, and thereby LMWH should be preferable to UFH. Many studies have shown the safety and efficacy of LMWH administration in prevention of pregnancy complications. These two drugs are not crossing the placenta and are not present in breast-milk. The treatment with LMWH or UFH should be recommended prenatally and for six weeks after delivery [47].

Because of their placental penetration, vitamin K antagonists do cause serious fetal malformation and they are contraindicated in the preconception period until the first trimester. Application of vitamin K antagonists is associated with fetal and maternal hemorrhage. For this reason, they are also contraindicated in the peripartum period.

Replacement of vitamin K antagonists with LMWH or UFH should happen before conception or when pregnancy is achieved [47,61].

It has been shown that the use of aspirin and heparin improves pregnancy outcome in women with previous obstetrical complications and APS. A prospective study that included 15 lactating women using low-dose aspirin showed no negative effects of aspirin therapy during lactation [47,61].

1.5.3. Treatment Methods in Graz

At the Department of Obstetrics and Gynecology, Medical University of Graz pregnant women with diagnosed thrombophilia are treated with weight-adjusted doses of LMWH.

The administration of LMWH should start before the 16th week of pregnancy. It is assumed that this early start of prophylaxis prevents both the occurrence of pregnancy-related venous thrombosis, and the occurrence of obstetric complications.

Pregnant women with diagnosed FVL-Mutation are treated with LMWH during pregnancy and in the puerperium.

Immunological disorders, such as SLE, and APS require the treatment with LMWH in combination with Aspirin.

Women with a history of previous obstetric complications, such as preeclampsia, eclampsia, and HELLP-Syndrome receive prophylaxis with aspirin [53].

2. Material and Methods

This thesis is based on a retrospective study that was conducted at the Department of Obstetrics and Gynecology, Medical University of Graz. The study was approved by Ethics Committee of Medical University of Graz (24-55 ex 11/12). Written explanation of the patients, as well as their written confirmation were not needed. All used informations for our study patient collective are treated strict confidentially.

This retrospective study was also developed with purpose to work out our research question that is defined as follows:" The administration of a heparin and/ or aspirin during pregnancy improves the maternal and fetal outcome of pregnant women with diagnosed FVL-Mutation".

The following variables were recorded for all 104 patients and their infants, if available:

1. Demographic data and pregnancy associated demographic data:
    - Maternal age
    - Gestational age at birth
    - Mode of delivery
    - Infant gender
    - Birth weight
2. Pre-existing diseases (e.g. venous thromboembolism, pre-existing hypertension, idiopathic-thrombocytopenic purpura, spontaneous pneumothorax, valvular heart disease)
3. Pregnancy-related complications (e.g. pregnancy-related venous thromboembolism, pre-eclampsia, eclampsia, and HELLP-Syndrome)
4. Fetal complications (e.g. intrauterine growth restriction and intrauterine fetal death)

The evaluation of maternal and fetal outcome of pregnant women with diagnosed FVL-Mutation is based on following parameters:

1. Maternal outcome parameters were:
    - Pregnancy- related venous thromboembolism
    - Occurring of preeclampsia, eclampsia and HELLP-Syndrome
    - Abruption Placentae
2. Fetal outcome parameters were:
    - Intrauterine fetal growth restriction
    - Intrauterine fetal death

Furthermore, in our study population we compared the maternal and fetal outcome of pregnant women, who were using any kind of antithrombotic therapy with those pregnant women, who were not using antithrombotic therapy.

2.1. Study population

Our study population was based on information from the Unit for management of hypertensive pregnancies of the Medical University of Graz. All pregnant women with a documented history of FVL-Mutation, who had been observed in this Unit between 2006 and 2012, were assessed.

Initially, our study included 106 patients. Two of them were excluded because of missing data, therefore, our study includes 104 patients.

Data of study population was selected from password-secured medical records, used at the Department of Obstetrics of Medical University of Graz such as, PIA/Viewpoint and OpenMedocs. Collected data were summarized in a Microsoft Excel table that was used as a database.

2.2. Analysis and Statistics

Statistical analysis was performed using Microsoft Excel 2010. We used Microsoft Excel 2010 to generate descriptive statistics that we illustrated due to charts, which were also created in Microsoft Excel 2010.

To analyze significant differences between our two study groups we used Chi-Square test (SPSS V21). P values < 0.05 were considered to be significant.

Odds ratio was used to quantify the effect of usage of antithrombotic drugs during pregnancy on the maternal and fetal outcome.

3. Results

3.1. Patient collective

All patients included in our study have a diagnosed FVL-Mutation. Other inclusion criteria for this study were treatment during pregnancy, as well as childbirth at the Department of Obstetrics of the Medical University of Graz. Two women were pregnant with twins, but we observed these women as women with single pregnancies, so that our study population includes 104 patients, and 104 infants.

The patients selected for this study were divided into two groups: the first group (n=82) consists of patients with diagnosed FVL-Mutation, treated by any kind of antithrombotic therapy during pregnancy; and the second group (n=22) includes patients with diagnosed FVL- Mutation without antithrombotic therapy during pregnancy. Among the first group we also examined which antithrombotic drug was used during pregnancy (LMHW, Aspirin or combination of both drugs). Fig. 8 is an overview of our study population.

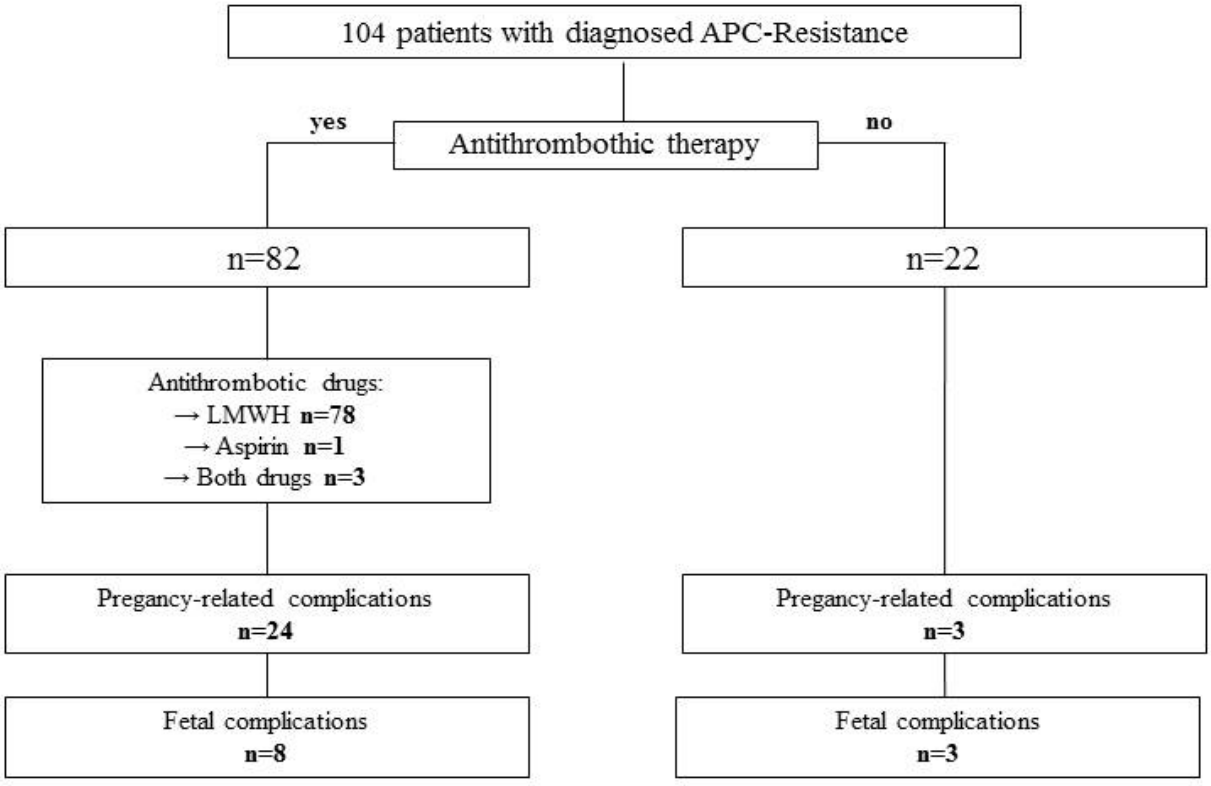

Figure 8: Patient collective

3.2. Demographic Data and Pregnancy Associated Demographic Data:

3.2.1. Maternal Age

Mean maternal age at time of delivery, was 29.08 ± 5.65, and it is ranging from 18 to 45 years.

For better insight into maternal age distribution at time of delivery, we divided our study population into 5-year age groups. The group 20 years and below includes 3 patients (2.88%). 23 patients (22.12%) belong to the group between 21 and 25 years. The largest age group is the age group 30 to 35 years with 35 patients (33.65%). The second largest age group is the age group 26 to 30 years, which includes 34 patients (32.69%). Older than 36 years are nine patients (8.65%). Figure 9 gives an overview over the maternal age among our study population.

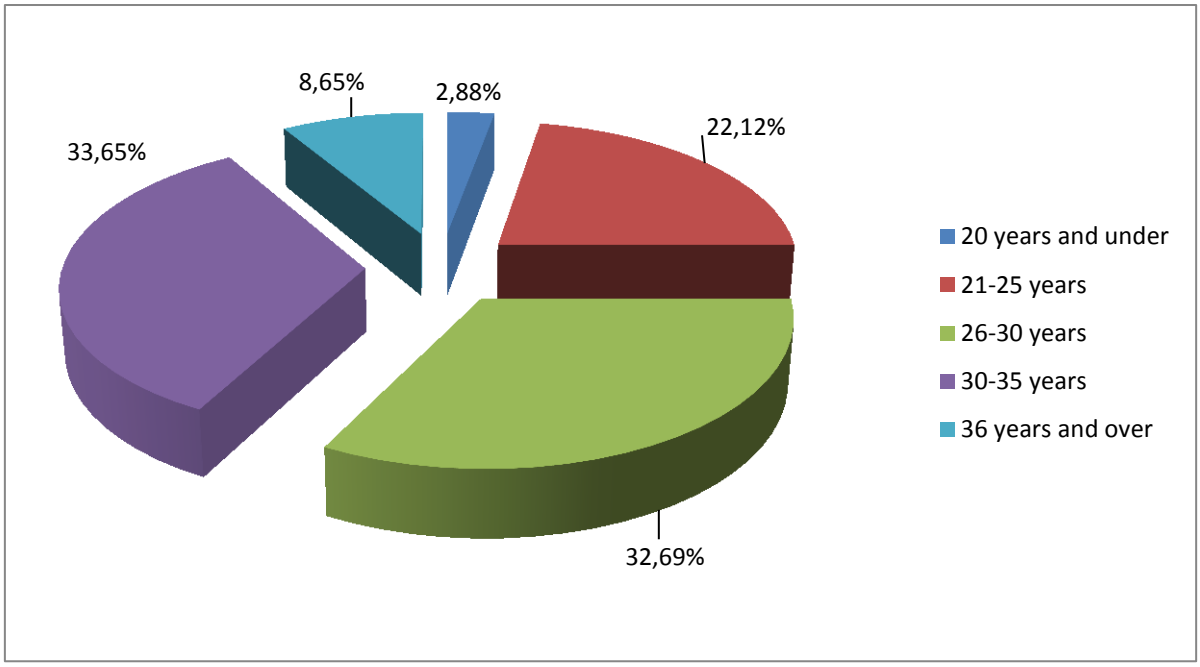

Figure 9: Percentage distribution of maternal age

3.2.2. Gestational Age at Birth

According to World Health Organisation (WHO), deliveries before the 37th week of gestation are defined as preterm. Preterm birth can be further subdivided into several groups. Deliveries between 32 and 37 weeks of gestation belong to the moderate to late preterm birth group. Deliveries between 28 and 32 weeks of pregnancy belong to the very preterm birth group. Deliveries before 28 weeks of gestation belong to the extremely preterm birth group. Preterm babies do not have sufficient time to develop in the womb and they can have serious problems in the postpartal period [62]. Term deliveries are deliveries after 37 weeks of gestation. Term deliveries could be also categorised by the gestational age at delivery into several groups [63]. Table 10 shows the classification of deliveries before and after 37 weeks of gestation.

Table 10: Subgroups of '(pre)term' deliveries; modified according to [62,63]

Classification of deliveries before 37 weeks of gestation	
extremely preterm	before 27 6/7 weeks
very preterm	28 0/7 weeks through 31 6/7
moderate to late preterm	32 0/7 weeks through 36 6/7
Classification of deliveries from 37 weeks of gestation	
Early term	37 0/7 weeks through 38 6/7
Full term	39 0/7 weeks through 40 6/7
Late term	41 0/7 weeks through 41 6/7
Post term	42 0/7 weeks and beyond

Gestational age at delivery of our study population, documented in pregnancy weeks, ranges from 27 to 42 weeks (mean 38.14 ± 2.96).

From a total of 104 patients, 22 women (21.15%) delivered before 37 weeks of pregnancy were completed. The moderate to late-preterm birth group included 18 births (81.82%). Three births (13.64%) belonged to the very-preterm birth group. One newborn (4.55%) was an extremely preterm baby. The number of women, who have their labor after 37th

week of gestation was completed, was 82 (78.85%), so that ´term` deliveries among our study population were more frequent than premature labor. The largest group of `term` deliveries is the full-term birth group with 57.32% (n=47). Nineteen births (32.17%) belong to the early-term birth group. The late-term birth group includes four births (17.07%). Just two of 82 women delivered their babies after 42nd week of pregnancy was completed (2.44%). Fig. 10 gives an overview over the frequency of occurrence of premature births and `term` deliveries, including all subgroups of these two groups.

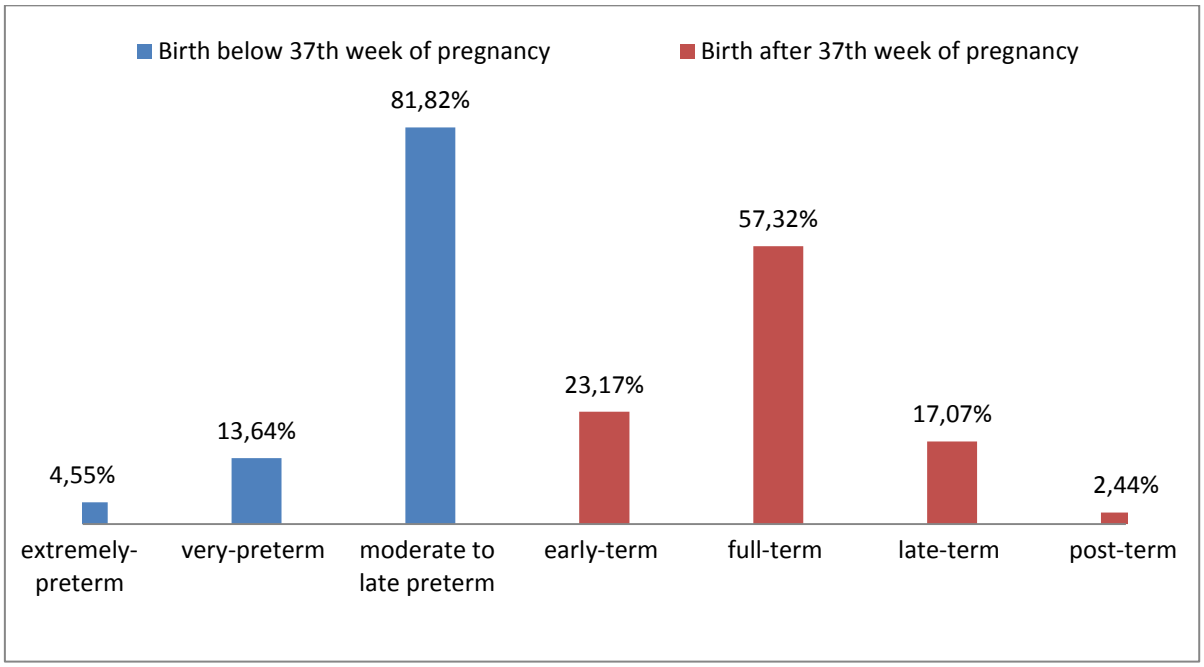

Figure 10: Percentage frequency of preterm and term birth subgroups

3.2.3. Mode of Delivery, Infant Gender and Birth Weight

Our study population includes 103 live births. The most common mode of delivery was vaginal delivery (70 patients - 67.31%). 34 (32.69%) patients underwent a caesarean section (c-section). We differentiated those patients, who underwent primary c-section, also called planned section n=16 (15.38%), from those, who underwent secondary c-section n=18 (17.31%). Reasons for secondary c-section were, for example: prolonged labor, fetal distress, maternal distress etc.

Table 11 shows the frequency of these three types of childbirth in absolute and relative terms in total and associated with fetal gender.

From total 104 born infants, 59 (56.73%) are male and 45 (43.27%) are female. One of the 70 vaginal deliveries was a stillbirth and it was a female fetus. Table 11 also shows the gender distribution of the infants.

Table 11: Frequency of relevant delivery types and gender distribution of the infants

Mode of delivery			**Infant gender**			
			Female		Male	
	n	%	n	%	n	%
vaginal delivery	70	67.31%	32	71.11%	38	64.41%
primary caesarean section	16	15.38%	5	11.11%	11	18.64%
secondary caesarean section	18	17.31%	8	17.78%	10	16.95%

Mean birth weight, documented in gram, was 3041.43±737.7, whereby, the lowest birth weight was 840g (stillbirth), and the highest one was 4572 g.

After delivery, newborn condition was evaluated due to Apgar score. To represent the condition of the newborns we analyzed Apgar score values ten minutes after their delivery. In our study population the Apgar score was recorded for 102 from a total of 104 infants. 94 infants (92.16%) had a 10min-Apgar of 10. Seven infants (6.86%) had Apgar scores lower than 10. One fetus (0.98%) was a stillbirth with a 10min-Apgar of zero.

3.3. Pre-existing Diseases and Pregnancy- related Complications

We also analyzed pre-existing diseases, as well as pregnancy complications. We found that 53 (50.96%) of 104 women had either disorders in medical history or pregnancy complications during the current pregnancy.

We divided twenty-six obtained cases (49.06%) with pre-existing diseases into two groups: (1) previous venous thromboembolism and (2) all other diseases. With 73.08% (n=19) the venous thromboembolism group is larger than the group that includes all other disorders (n=7, 26.92%). In this second group of previous disorders, pre-existing hypertension appeared in 3 cases (11.54%). Two patients (7.69%) had idiopathic-thrombocytopenic purpura. One woman (3.85%) had spontaneous pneumothorax in her medical history and one woman (3.85%) had a valvular heart disease.

We also divided patients with pregnancy complications (n=27; 50.94%) into two groups: (1) pregnancy-related venous thromboembolism, and (2) all other pregnancy complications.

Two of 27 women (7.41%) developed venous thromboembolism during current pregnancy. Preeclampsia occurred in 5 pregnancies (18.52%). Other hypertension associated pregnancy complications, such as eclampsia and HELLP-Syndrome appeared in two cases (7.41%). One case (3.7%) of placental abruption was described. Gestational diabetes mellitus (GDM) was with 48.15% (n=13) the most common pregnancy complication. Four women (14.81%) suffered from pregnancy-induced hypertension.

Figure 11 illustrates the frequency of pre-existing and pregnancy-related disorders in our study population.

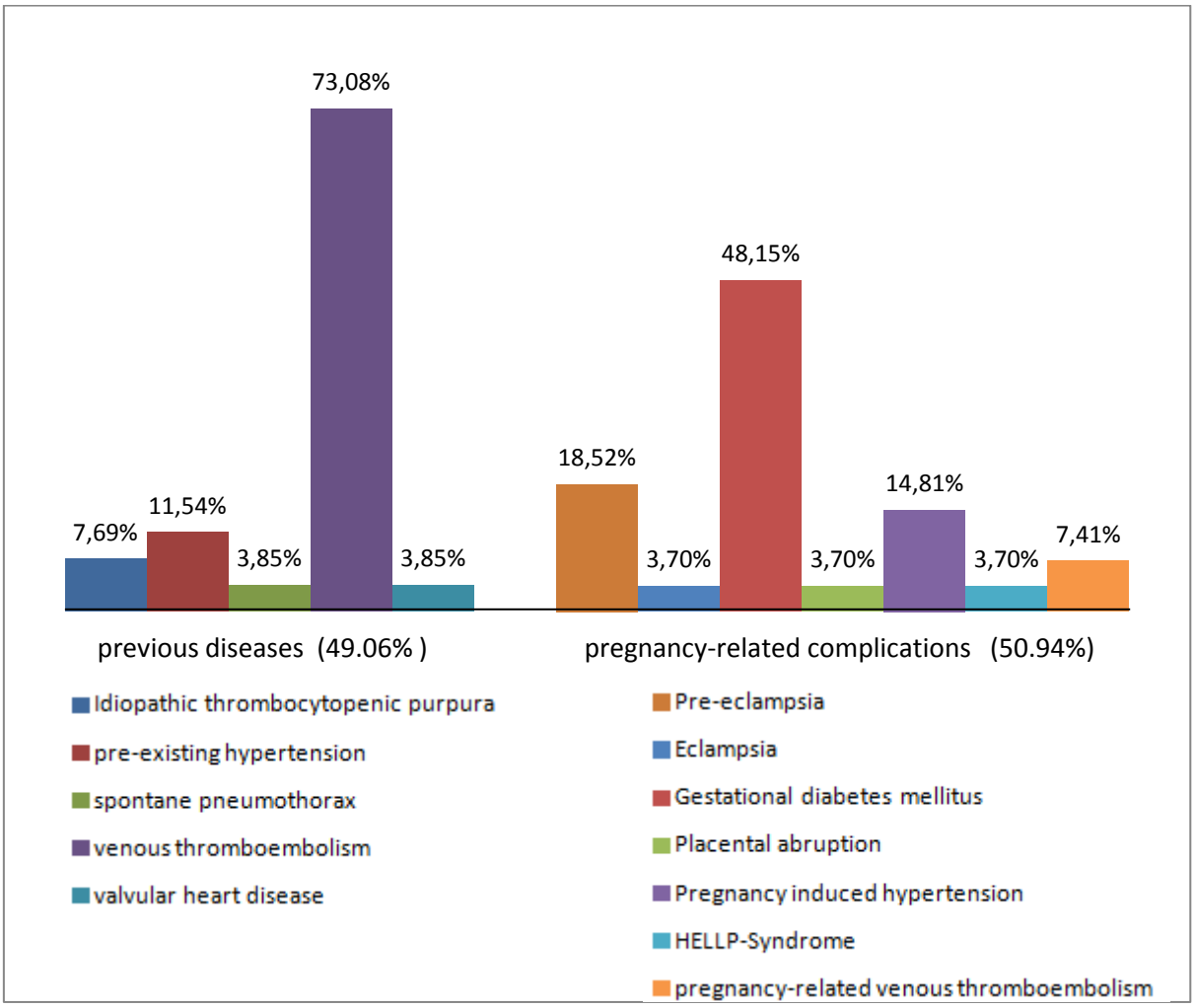

Figure 11: Percentage frequency of pre-existing diseases and pregnancy-related complications

3.4. Fetal Complications

This study includes 104 infants. Eleven of 104 babies (10.58%) developed fetal complications. 10 infants (90.91%) developed intrauterine growth restriction (IUGR). Intrauterine fetal death (IUGD) was confirmed in one pregnancy (9.09%). The rest of the infants (n=93, 89.42%) did not develop any fetal complications during pregnancy.

Figure 12 gives an overview over the occurrence of fetal complications among our study infants

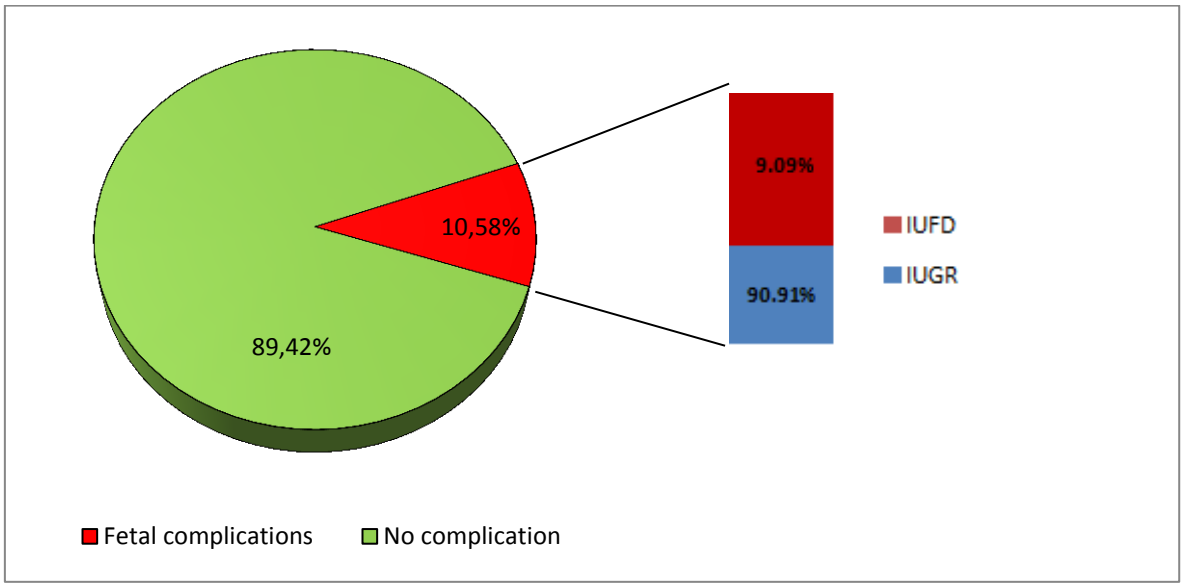

Figure 12: Percentage frequency of fetal complications

3.5. Antithrombotic Therapy during Pregnancy and Pregnancy-related Complications

From a total of 104 women, 82 (78.85%) received antithrombotic medication during pregnancy. 24 (29.27%) of them, developed pregnancy complications.

With 95.12% (n=78), the most common antithrombotic drug applied was LMWH. One woman (1.22%) received Low Dose Aspirin. Three of 82 women (2.88%) received LMWH as well as Low Dose Aspirin.

Among women with LMWH as antithrombotic prophylaxis during pregnancy (n=78, 95.12%), we found 20 cases of pregnancy-related complications. With 55% (n=11) gestational diabetes mellitus was the most common pregnancy related complication. 4 women (20%) who used LMWH as antithrombotic drug developed pregnancy-induced hypertension. Pregnancy-related venous thromboembolism occurred in one pregnancy

(5%) that was treated with LMWH. One case of HELLP-Syndrome (5%) and 3 cases of preeclampsia (15%) were documented in this group. Eclampsia, as well as placental abruption was not documented among women who were treated only with LMWH.

One woman (1.22%) of all (82) treated women received low-dose aspirin as antithrombotic prophylaxis during pregnancy. Her pregnancy was associated with pre-eclampsia, and she also suffered placental abruption.

Among women who received LMWH as well as Aspirin, one developed preeclampsia and another one developed eclampsia during pregnancy.

Figure 13 gives an overview over the frequency of pregnancy complications associated with therapy-patterns, which were used during pregnancy.

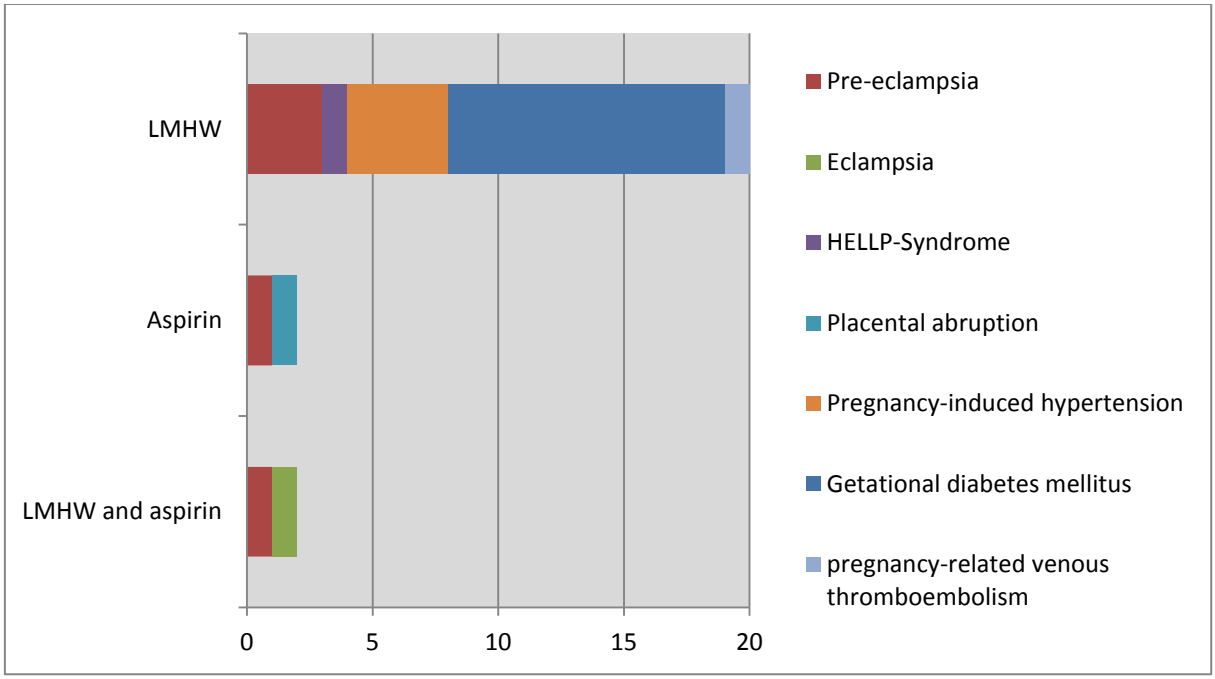

Figure 13: Percentage frequency of pregnancy complications

Among women, who did not use antithrombotic drugs (n=22, 21.15%), 3 of them (13.64%) developed pregnancy complications. Gestational diabetes mellitus occurred with a frequency of 66.67% (n=2). One case of pregnancy-related venous thromboembolism (33.33%) was documented among women who developed pregnancy complications and did not receive antithrombotic prophylaxis.

Application of antithrombotic medication reduces pregnancy complications compared to pregnancies without medication, though the results were not significant (p=0.138).

The table 12 gives an overview over all parameters that we used to represent the maternal outcome among our study population. This table also represents the maternal outcome depending on the usage or no usage of the antithrombotic prophylaxis and it also represents the maternal outcome depending on the antithrombotic drugs, which were used as treatment.

Table 12: Maternal Outcome

			Maternal outcome								
			For our study relevant pregnancy-related complications						Other pregnancy-related complications		
			Pregnancy-related venous thromboembolism	**Hypertensive disorders**			**Placenta abruption**	total	GDM	Pregnancy-induced hypertension	total
				PE	**E**	**HELLP-Syndrome**					
Therapy n=82 78.84%	**LMWH**	n=78 95.12 %	n=1, 20% (5%)	n=3, 60% (15%)	/	n=1, 20% (5%)	/	**n=5**	n=11, 73% (55%)	n=4, 27% (20%)	n=15
	Low-dose aspirin	n=1 1.21%	/	n=1, 50%	/	/	n=1, 50%	**n=2**	/	/	/
	LMWH and low-dose aspirin	n=3 3.65%	/	n=1, 50%	n=1, 50%	/	/	**n=2**	/	/	/
total			n=1	n=5	n=1	n=1	n=1	**n=9**	n=11	n=4	n=15
without therapy n=22 21.15%			n=1, 100% (33.33%)	/	/	/	/	**n=1**	n=2, 100% (66.67%)	/	n=2
∑			**n=2**	**n=5**	**n=1**	**n=1**	**n=1**	**n=10**	n=13	n=4	n=17

3.6. Antithrombotic Therapy during Pregnancy and Fetal Complications

In our study population, fetal complications occurred in eleven pregnancies. Eight (9.76%) from total of 82 pregnancies treated with antithrombotic medication were associated with fetal complications. Three cases (13.64%) with fetal complications were documented among those women who did not receive antithrombotic drugs.

Among women who received LWMH or/and low-dose aspirin only documented fetal complication was IUGR. From a total of 78 women treated with LWMH, IUGR occurred in 5 pregnancies (6.41%). One woman was treated only with low-dose aspirin and her pregnancy was also associated with IUGR. Among three women who received both LWMH and low-dose aspirin, IUGR occurred in two pregnancies (66.67%).

From eight cases associated with fetal complications among women who received any kind of antithrombotic prophylaxis, five cases (62.5%) were associated with LWMH, one case was associated with low-dose aspirin, and the remaining two cases (25%) were associated with administration of both LWMH and low-dose aspirin. Among 22 pregnancies without antithrombotic therapy, fetal complications occurred in three cases (13.64%). One of them (33.33%) was an intrauterine fetal death and the other 2 (66.67%) were IUGRs.

With an OR of 0.685 (95% CI 0.166 -2.831) the probability for occurrence of fetal complications among infants of women using an antithrombotic therapy is 1.458 times higher compared to infants of women who were not using an antithrombotic therapy during pregnancy.

Table 13 gives an overview over the frequency of fetal complications depending on the therapy, which was used during pregnancy.

Table 13: Percentage frequency of fetal complications (treated versus non treated women)

	Therapy			Without Therapy
Complications	**NHM**	**ASS**	**Both**	
IUGR	62.5%	12.5%	25%	66.67%
IUFD	0%	0%	0%	33.33%

The table 14 gives an overview over all parameters that we used to represent the fetal outcome among our study population. This table also represents the fetal outcome depending on the maternal usage or no usage of the antithrombotic prophylaxis and it also represents the fetal outcome depending on the antithrombotic drugs, which were used as treatment.

Table 14: Fetal Outcome

			Fetal outcome		
			IUGR	**IUGD**	total
Therapy n=82, 78.84%	**LMWH**	n=78	n=5	/	n=5
	Low-dose aspirin	n=1	n=1	/	n=1
	LMWH and low-dose aspirin	n=3	n=2	/	n=2
total			**n=8**	/	**n=8**
Without therapy n=22, 21.15%			n=2	n=1	n=3
∑			n=10	n=1	n=11

4. Discussion:

Our study collective included all patients with diagnosed FVL-Mutation, who have delivered and who were eventually treated at the Department for Obstetrics of Medical University of Graz in the time between 2006 and 2012.

This retrospective study evaluates pregnancy outcome of women with Factor V Leiden-Mutation. Therefore we analyzed following parameters:

- Pregnancy- related venous thromboembolism
- Occurring of preeclampsia, eclampsia and HELLP-Syndrome
- Abruption placentae
- Intrauterine fetal growth restriction
- Intrauterine fetal death.

Furthermore, this study was conducted for the purpose of showing if application antithrombotic drugs has an impact on pregnancy outcomes and thus, we want to point out the eventually need for adjustment of the antithrombotic therapy in women with diagnosed FVL-Mutation. This was the reason why, we divided our study population (104) into two groups: (1) with antithrombotic drugs treated women (n=82, 78.84%), and (2) women who have not received antithrombotic drugs during current pregnancy (n=22, 21.15%). It is also important to mention, that in our study two women were pregnant with twins, but we observed these women as a women with single pregnancies and our study population includes 104 patients.

To present the occurrence of pregnancy-related complications depending on the therapy that was applied during current pregnancy, we divided the group of treated women (n=82, 78.84%) into 3 groups: (1) women, who received LMWH (n=78, 95.12%), (2) women, who received only low-dose aspirin (n=1, 1.21%), and (3) women, who received both of these antithrombotic drugs (n=3, 3.65%). After we divided our study population we looked after following five pregnancy-related complications: (1) pregnancy-related venous thromboembolism, (2) pre-eclampsia, (3) ecplampsia, (4) HELLP-Syndrome, and (5) placental abruption.

4.1. Maternal outcome:

To represent maternal outcome of our study population we analyzed the data of 104 patients.

Mean maternal age at time of delivery, given in years, was 29.08 ± 5.65.

Gestational age at delivery of our study population, documented in pregnancy weeks, ranges from 27 to 42 weeks (mean 38.14 ± 2.96). With 78.85% (n=82) were 'term' deliveries among our study population more frequently as the premature labor (n=22, 21.15%).

From a total of 104 obtained cases, for 53 of them (50.96%) we found either positive medical history for pre-existing diseases (n=26, 49.06%) or we found pregnancy complications in current pregnancy (n=27, 50.94%). Figure 10 gives an overview over prevalences of all previous diseases and pregnancy complications among our study population. It is important to mention, that we did not look for association between pre-existing diseases and the occurrence of pregnancy complications in current pregnancy. In the group of previous diseases is venous thromboembolism the most common disease (n=19, 73.08%). Whereby, the most frequently pregnancy complication documented in delivery charts was GDM with 48.15% (n=13).

Table 12 gives an overview over all parameters that we used to represent the maternal outcome among our study population.

Compared to non-pregnant women, pregnant women have approximately a 3-to 20-fold increased risk for venous thromboembolism. Risk for VTE during pregnancy increases even more if other factors are present, such as thrombophilia [25,47,49].

A meta analysis by Biron- Andreani et al. combined results from five cohort studies and six case-control studies. The pooled odds ratios of pregnancy- related venous thromboembolism in women carrying the FVL is shown in table 15 [50].

A systematic review by Robertson et al. included seventy-nine studies and examined the risk for pregnancy- related venous thromboembolism in women carrying the Factor V Leiden Mutation. Odds ratio for pregnancy- related venous thromoembolism among FVL homozygous carries and odds ratio of pregnancy-related venous thromboembolism among FVL heterozygous carriers can be also seen in the table 15 [51].

Table 15: Pooled odds ratios of pregnancy- related VTE in FVL- positive women; modified according to [50,51]

		Pregnancy- related VTE	
		OR	95% CI
A meta analysis by Biron-Andreani et al., 2006	Cohort studies	8.6	5.9-12.6
	Case-control studies	4.5	1.8-11.0
A systematic review by Robertson et al., 2005	FVL-homozygous	34.40	9.86-120.05
	FVL-heterozygous	8.32	5.44-12.70

VTE = venous thromboembolism; OR = odds ratio; CI = confidence interval; FVL = factor V Leiden

As can be seen in the table 12, just two women in our study collective (n=104, 1.92%) have suffered from VTE in current pregnancy, whereat, one case was documented among women using antithrombotic drugs (n=82, 1.21%) and other one occurred among women, who were not received antithrombotic drugs (n=22, 4.54%).

The study by Bironi-Andreani et al. and the study by Robertson et al. represented the increased risk for occurrence of VTE in women carrying FVL-Mutation without reference to treatment. Compared to these results, we cannot say that the occurrence of VTE increases with the presence of FVL-Mutation, because we do not have a control group (women without FVL-Mutation). With frequency of 2%, in our study VTE occurred rarely compared to literature.

Placental-mediated pregnancy complications (PmC), e.g. pre-eclampsia, placental abruption, IUGR, and IUFD are the most common reasons for maternal and fetal mortality and morbidity. These PmC occur more often among women with thrombophilia compared to healthy women. Thus, it is logical to assume that thrombophilia plays an important role in pathogenesis of these pregnancy complications. It cannot be said that inherited thrombophilia causes pregnancy complications, but it is one of risk factors that is associated with pregnancy complications [64].

Hypertensive disorders (preeclampsia, eclampsia, HELLP-Syndrome) were documented in 7 pregnancies. All of them occurred among women using antithrombotic drugs (n=82) (Table 12).

A systemic review by Robertson et al. reported about significant association between preeclampsia and heterozygous factor V Leiden without reference to treatment. The pooled odds ratio was estimated to be 2.19 (95% confidence interval (CI) 1.96-3.27) [51].

Table 16 shows an overview of odds ratios (ORs) of preeclampsia in FVL- positive women.

Table 16: Odds ratios of preeclampsia in FVL (homozygous and heterozygous)-positive women; modified according to [22]

		Preeclampsia	
Study	**Country**	**OR**	**95% CI**
Clark et al., 2008	United Kingdom	1.27	0.39-4.08
Dudding et al., 2008	United Kingdom	1.48	0.88-2.46
Karakantza et al., 2007	Patras, Greece	1.62	0.9-29.52
Lindquist et al., 2006	Malmo, Sweden	1.23	0.48-3.17
Murphy et al., 2000	Dublin, Ireland	1.59	0.09-28.26

OR = odds ratio; CI = confidence interval

Among our study collective (n=104) preeclampsia occurred in 5 pregnancies (4.8%), whereat, all cases were documented among women who were using antithrombotic drugs (n=82, 6.1%) (Table 12). Compared to results of the study by Robertson et al., occurrence of preeclampsia in our study is infrequent.

The study by Schlembach et al. showed a twofold elevated prevalence of maternal FVL in HELLP patients compared to the control group, which consisted of women without HELLP-Syndrome [55]. Furthermore, the study by Benedetto et al. reported about significant association between FVL and HELLP-Syndrome [56].

In our study population HELLP-Syndrome occurred in just one pregnancy (0.96%), namely, in group of patients treated with antithrombotic drugs (table 12).

By lacking of control group (women without FVL-Mutation, who are suffering from HELLP-Syndrome), prevalence of HELLP-Syndrome cannot be compared and with a frequency of 1% it cannot be said that the presence of FVL-Mutation significantly increases the risk to develop HELLP-Syndrome.

Rodger et al. analyzed data from five studies that were reporting about association between FVL-Mutation and placenta abruption. The absolute risk of placenta abruption among women with diagnosed FVL-Mutation was 1.3% compared to 0.9% for women without FVL-Mutation. The pooled OR was estimated to be 1.85 (95% CI 0.92-3.70) [22].

A systematic review by Robertson et al. included seven studies to evaluate the association between thrombophilia and placental abruption without reference to treatment. Increased risk for placental abruption is associated with thrombophilia of any origin, but significant association is only detected among women with heterozygous FVL (OR 4.70, 95% CI 1.13-19.59) [51].

Only one woman (0.96%) in our study population, who was treated with antithrombotic drugs suffered from placental abruption (table 12).

In contrast to results of previous studies, the results of our study (prevalence of 1%) do not show that the risk for placental abruption is increased, if the woman is suffering from FVL-Mutation, on the one hand, because this pregnancy complication occurred just once in our study population, and on the other hand, because we did not have the same control group (women without FVL-Mutation).

UFH, LWMH and low-dose aspirin have long been used as prophylaxis against pregnancy complications [64].

Heparins own, beside anticoagulation effects, also anti-inflammatory effects that positively impact placentation independent of type of thrombophilia. Even if heparins may reduce the occurrence of PmC, their unlimited administration among women with known inherited thrombophilia needs to be investigated [64,65].

At the Department of Obstetrics and Gynecology at Medical University of Graz all pregnant women with diagnosed thrombophilia are treated with weight-adjusted LMWH. That is the reason why LMWH is the most common drug used against thromboembolic events and pregnancy complications among our study population (n=78, 95.12%).

With 55% (n=11) gestational diabetes mellitus was the most common pregnancy complication among women who were treated only with LMWH. Four women (20%) with LMWH as antithrombotic drug developed pregnancy-induced hypertension (Table 12). GDM and pregnancy-induced hypertension were not relevant parameters in this study. Those variables are only described in our statistical analysis.

As one of five relevant pregnancy-related complications in our study, pre-eclampsia was the most common complication among women treated with LMWH as antithrombotic

therapy (60%). In this study group we documented our only case of HELLP-Syndrome (20%).

Just one case of pregnancy-related venous thromboembolism (20%) was documented among women using LMWH as therapy (Table 12).

Only one woman (1.22%) among 82 treated women treated with antithrombotic drugs received low-dose aspirin as prophylaxis. During her pregnancy, she developed pre-eclampsia. She also suffered from placental abruption. This pregnancy and thereby this study group was the only one that was associated with placental abruption.

Due to administration of LMWH in combination with low-dose aspirin the recurrence of PmC can be improved towards sole low-dose aspirin or LMWH application [64,66,67].

The study by de Varies et al. reported also about the reduction of risk for recurrence of PmC from 9% to 0%, when LMWH is added to low-dose aspirin [64].

The results from study by Gries et al. showed that the risk to develop severe pre-eclampsia decreases from 7% to 1%, when LMWH is administrated with low-dose aspirin [65].

Among those women (n=3, 3.65%), who received both drugs as antithrombotic prophylaxis we documented one case of pre-eclampsia and one case of eclampsia in two different pregnancies. One pregnancy in this study group was without any of pregnancy-related complications.

Among our study population (n=104) 22 women (21.15%) did not receive antithrombotic drugs. 13.64% (n=3) developed pregnancy-related complications. One pregnancy (33.33%) was associated with pregnancy-related venous thromboembolism, as one of the five for our study relevant pregnancy-related complications. Other two pregnancies (66.67%) were associated with GDM that was not relevant for our study.

The retrospective cohort study by Kupferminc et al. showed that the usage of LMWH significantly reduces the occurrence of maternal complications among women with thrombophilia when compared with control group (women, who have not received LMWH). Hence, administration of LMWH reduces the occurrence of severe pre-eclampsia by 16.4% [62].

In our study, with Chi-Square Test (p=0.138) it cannot be proved that the use of antithrombotic drugs significantly reduces the occurrence of pregnancy complications compared with women who were not using an antithrombotic prophylaxis, but, an OR of 2.621 (95% CI 0.709-9.686) shows that the probability of the occurrence of pregnancy complications among women using an antithrombotic therapy is 2.621 times lower compared to women without antithrombotic treatment during pregnancy.

4.2. Fetal outcome:

To represent fetal outcome we analysed data of 104 infants.

Our study population includes 103 live births. With 67.31% (n=70), vaginal birth was the most common mode of delivery. 34 infants (32.69%) were delivered by caesarean section.

From total 104 born infants, 59 babies (56.73%) are male, whereby, 38 of them (64.41%) were delivered vaginally and the rest (n=21, 35.59%) were delivered by c-section. The female group includes 45 infants (43.27%). 32 of them (71.11%) were delivered vaginally and the rest (n=13, 18.89%) were delivered by c-section. One case in the female group was stillbirth. This case of stillbirth was also the only one in our study.

Mean birth weight, documented in gram, is 3041.43±737.7, whereby, the lowest measured birth weight is 840g (stillbirth), and the highest one is 4572 g.

To represent the condition of the newborn babies we analyzed the Apgar score values measured at ten minutes after their delivery. In our study population the Apgar score was recorded for 102 from a total of 104 infants. 94 infants (92.16%) showed Apgar score of 10. Seven infants (6.86%) had Apgar score lower than 10 points. One fetus (0.98%) was a stillbirth, which is why we have one Apgar score value of 0 points.

Table 14 gives an overview over all parameters that we used to represent the fetal outcome among our study population.

The association between thrombophilias and IUGR was described in a systemic review by Robertson et al. that included five studies. The results showed an increased IUGR risk in pregnant women with thrombophilias and without reference to treatment, namely, an OR of 4.64 (95% CI 0.19-115.68) for homozygous FVL women and an OR of 2.68 (95% CI 0.56-12.13) for heterozygous FVL women [51].

Due to data from twelve case-control studies and four cohort studies, a meta-analysis by Facco et al. described a significant association between FVL and IUGR without reference to treatment (OR 1.23, 95% CI 1.04-1.44) (Table 17) [58].

Table 17: Odds ratios of intrauterine growth restriction in FVL (homozygous and heterozygous)-positive women; modified according to [58]

		IUGR	
Case –control studies	**Country**	**OR**	**95% CI**
Martinelli et al., 2001	Italy	6.87	1.41-33.55
Agorastos et al., 2002	Greece	6.00	1.20-30.10
Franchi et al., 2004	Italy	1.38	0.22-8.53
Verspyck et al., 2004	France	2.58	0.80-8.36
Jarvenpaa et al., 2006	Finland	4.49	0.83-24.18
Karakantza et al., 2008	Greece	0.70	0.04-12.36
Özbek et al., 2008	Turkey	0.29	0.01-6.17
Cohort studies	**Country**	**OR**	**95% CI**
Lindquist et al., 1999	Sweden	0.93	0.46-1.88
Murphy et al., 2000	Ireland	1.80	0.10-32.20
Nurk et al., 2006	Norway	1.20	1.00-1.45

IUGR = intrauterine growth restriction; OR = odds ratio; CI = confidence interval

In order to evaluate the association between FVL and pregnancy loss, Robertson et al. used data from twenty-five studies for early pregnancy loss and data from five studies for late pregnancy loss. Their systemic review showed a significant association for pregnancy loss among FVL women and without reference to treatment [51]. The pooled odds ratios for pregnancy loss in women with FVL (homozygous and heterozygous) are presented in table 18.

The study by Middeldorp S. showed the significant association for IUGD in women carrying FVL-Mutation and without reference to treatment due to following two ORs: (1) OR of 3.3 (95% CI 1.8-5.8), and (2) OR of 2.1 (95% CI 1.1-3.9) [23].

Table 18: Pooled odds of abortions in FVL- positive women; modified according to [51]

		OR	**95% CI**
Early pregnancy loss	FVL-homozygous	2.71	1.32-5.58
	FVL-heterozygous	1.68	1.09-2.58
Late pregnancy loss	FVL-heterozygous	2.06	1.10-3.86

VTE = venous thromboembolism; OR = odds ratio; CI = confidence interval; FVL = factor V Leiden

As can be seen in table 14, 11 infants (10.58%) of 104 babies in our study suffered either IUGR or IUGD. IUGD was confirmed in one pregnancy (9.09%). 10 infants (90.91%) developed IUGR as fetal complications. 8 (9.76%) of them occurred at infants of treated women (n=82). From these 8 cases, five cases (62.5%) were associated with LWMH, one case was associated with low-dose aspirin, and the remaining two cases (25%) were associated with administration of both LWMH and low-dose aspirin. However, the only fetal complication, infants of treated women developed, was IUGR.

Compared to results of the study by Robertson et al., the study by Facco et al. and the study by Middeldorp S., our study cannot show the significant association either for IUGR or for IUGD among women with FVL-Mutation.

Among 22 women (21.15%) who did not receive antithrombotic therapy during pregnancy, fetal complications occurred in three cases (13.64%). Whereby, in one pregnancy (33.33%) appeared intrauterine fetal death as fetal complication (that was the only case of IUGD in our study) and in other 2 pregnancies (66.67%) we found IUGR as fetal complication (Table 14).

The retrospective cohort study by Kupferminc et al. showed that the usage of LMWH significantly reduces the occurrence of fetal complications among women with thrombophilia compared to control group of women, who did not receive LMWH. Administration of LMWH reduces the occurrence of IUGR by 18.7% [62].

In our study, with Chi-Square Test (p=0.276) it cannot be proved that the use of antithrombotic drugs significantly reduces the occurrence of fetal complications compared with infants of women who were not using an antithrombotic prophylaxis, but, an OR of 0.685 (95% CI 0.166 -2.831) shows that the probability for the occurrence of fetal complications among infants of women using an antithrombotic therapy is 1.458 times higher compared to infants of women, who were not using an antithrombotic therapy during pregnancy.

Limitations of this study are: small case of numbers included in study, different number of subjects in study groups, unequal distribution of the art of the treatment, as well as the fact that data used for this study were collected retrospectively.

5. Conclusion

This retrospective study evaluates maternal and fetal outcome of women with FVL-Mutation dependent on application or not of antithrombotic prophylaxis, on the one hand, and on the other hand, dependent on manner of antithrombotic therapy.

Pregnancy complications appeared among treated women, as well as among not treated women. Among treated women, the most common maternal complication was pre-eclampsia and the most common fetal complication among infants of treated women, as well as among infant of not treated women was IUGR. Among treated women we found one case of placental abruption. This was the only one in our study population. From a total 22 not treated women, only one woman has suffered an IUGD. None of the not treated women suffered from pre-eclamsia, but one case of pregnancy-related venous thromboembolism was documented. Occurrence of pregnancy complications among our study population is very diverse.

Limitations of this study were the unequal number of subjects in the main groups (application or not of antithrombotic drugs), as well as unequal distribution of the treatment-patterns (the use of LMWH/low-dose Aspirin/both LMWH and low-dose Aspirin).

With p=0.138 for maternal complications and p=0.276 for fetal complications it cannot be proved that the application of antithrombotic drugs significantly reduces the occurrence neither of maternal nor of fetal complications compared with women, who were not using an antithrombotic prophylaxis. OR for maternal complications for women with antithrombotic prophylaxis compared with women without, was 2.621(95% CI 0.709-9.686). Respective fetal complications, OR was 0.685 (95% CI 0.166-2.831) for infants of women, who received antithrombotic prophylaxis compared with infants of women, who did not use an antithrombotic prophylaxis. Hence, our study has showed that the usage of antithrombotic drugs improves maternal outcome, but it has a negative impact on the fetal outcome.

Conduction of further trials in order to compare pregnancy outcome of several treatment methods with each other and to adjust the antithrombotic therapy in women with diagnosed FVL-Mutation seems to be necessary.

6. References

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