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SUMMARY

Authors name and surname; Amanda Cathrine Vest.

Research title: Intrinsic coagulation pathway disorders and laboratory diagnostics correlations.

Aim of study: The aim was to evaluate the contribution of routine and specialized coagulation laboratory testing to clinical decision making in suspected intrinsic coagulation disorders.

Objectives: to test and analyze routine coagulation test (aPTT and PT) results and their correlation with clinical and anamnestic data, to analyze the correlation between routine (aPTT) and specialized (factors VIII, IX, XI, XII activity) coagulation tests and to analyze the prevalence of coagulation changes and their differences in different age and gender groups.

Study participants: Patients used in this study had been consulted in outpatient department and in departments of the hospital of Lithuanian University of Health Sciences, Kauno Klinikos from January to December of 2016. The patients’ chosen for the study had routine coagulation test and specialized factor test results. 99 patients in total were included in this study. Analysis of demographic, clinical and laboratory data was done.

Methodology: For this study venous blood from patients was used, from the blood samples routine coagulation (aPTT and PT) and specialized clotting tests (FVIII, FIX, FXI and FXII) activity was measured. These measurements were done using the coagulation analyzer Compact Max (Diagnostica Stago, France).

Results: In this study it was determined that there is significant difference in the results of coagulation screening testing of patients with different clinical data. Patients with clear clinical indications had strongly significant prolongation of aPTT. It was also found that there is no significant difference in the prevalence of males and females affected by coagulation disorders included in this study. The results showed that there was equal distribution in the prevalence of affected patients in different age groups. It was determined that there is significant correlation between changes of aPTT and factors VIII and XII, while there is no significant correlation between aPTT changes and factors IX and XI. The analysis of overall prevalence of intrinsic factor activity changes in relation to gender and age of patient showed no significant difference in prevalence of disorders of all four intrinsic coagulation factors regardless of gender or age.

Conclusions:

1. The correlation between aPTT test results with clinical and anamnensis data differs depending on relevance of the data. In patients with clear indications, active bleeding, for screening coagulation tests the correlation with clinical status was strong and significant. With these results we
can conclude that in combination with a good clinical evaluation, screening tests of coagulation disorders is a valuable adjunct to diagnosing coagulation disorder.

2. It was determined that the correlation between routine and specialized coagulation tests was depending on coagulation disorder type. There was a significant correlation between aPTT value with the activity of FVIII and FXII, while there was no correlation between aPTT value with the activity of FIX and FXI. aPTT usage for the screening of intrinsic pathway coagulation disorders related to factor deficiencies is reliable.

3. It was found in this study that the overall prevalence of coagulation changes did not differ in the two age groups or between male and female gender.

**Keywords**

Intrinsic coagulation pathway disorders, activated partial thromboplastin time, prothrombin time, coagulation, bleeding.
CONFLICTS OF INTEREST

The author reports no conflicts of interest.

CLEARANCE ISSUED BY THE ETHICS COMMITTEE

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Biomedical research name: Intrinsic coagulation pathway disorders and laboratory diagnostics correlation.
Number: BEC-MF-455
Date: 2016-05-02
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>aPTT</td>
<td>Activated partial thromboplastin time</td>
</tr>
<tr>
<td>PT</td>
<td>Prothrombin time</td>
</tr>
<tr>
<td>df</td>
<td>Degree of freedom</td>
</tr>
<tr>
<td>FFP</td>
<td>Fresh frozen plasma</td>
</tr>
<tr>
<td>FII–FXIII</td>
<td>Coagulation factor II – coagulation factor XIII</td>
</tr>
<tr>
<td>FIIa–FXIIIa</td>
<td>Activated coagulation factor II – activated coagulation factor XIII</td>
</tr>
<tr>
<td>HMWK</td>
<td>High molecular weight kininogen</td>
</tr>
<tr>
<td>SPA</td>
<td>Stago Prothrombin Assay</td>
</tr>
<tr>
<td>TF</td>
<td>Tissue factor</td>
</tr>
<tr>
<td>tPA</td>
<td>Tissue-type plasminogen activator</td>
</tr>
<tr>
<td>uPA</td>
<td>Urokinase-type plasminogen activator</td>
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INTRODUCTION

Bleeding disorders are a group of disorders that share a common pathology: the inability to form a proper blood clot. They are usually characterized by prolonged bleeding during or after events where bleeding risk may be increased such as trauma and surgery.

There are 13 different clotting factors in the human circulation, if any of them are decreased or defective it affects the blood clotting process. The affection can be of mild, moderate or severe degree [1]. The most common type of intrinsic bleeding disorder is Hemophilia, which affects about 1 or every 5000 male births. There are two main types of hemophilia, A and B. Hemophilia A is about four times more common than hemophilia B [2]. Other types of intrinsic bleeding disorders, deficiency of factors XI and XII, are called rare bleeding disorders and as the name indicates they occur at a lesser rate.

The problem of diagnosing patients with bleeding disorder is that clinical symptoms may vary widely [3]. The symptoms range from asymptomatic to excessive bruising and unexplained nosebleeds and even bleeding into joints and tissue causing long-term complications. A careful history is necessary for patients’ where there is suspicion of bleeding disorder. It is the first step in recognizing a bleeding as pathological, although the sensitivity and specificity of clinical features is very limited.

The most accurate way to diagnose bleeding disorders is by taking venous blood from the patient and measure the time for coagulation in the laboratory. There is a widely used screening test, aPTT and PT, which allow medical staff to identify patients with coagulation pathologies. These screening tests may not be a hundred percent accurate and therefore they should not be relied upon to confirm or disregard a diagnosis of bleeding disorder, especially in patients with a clear history of bleeding. The next step in the diagnosis is to use venous blood from the patient and looking for the number of specific clotting factor in the laboratory. This is a more specific way to diagnose a factor deficiency and type of bleeding disorder. Finding and diagnosing patients with bleeding disorders is important to give patients proper treatment and prophylaxis.

The aim of this research is to evaluate the contribution of routine coagulation testing, aPTT and PT, as well as specialized coagulation testing to clinical decision making.
AIM AND OBJECTIVES

Aim: The aim of this study was to evaluate the contribution of routine and specialized coagulation laboratory testing to clinical decision making in suspected intrinsic coagulation disorders.

Objectives:
1. to test and analyse routine coagulation test (aPTT and PT) results and their correlation with clinical and anamnesis data;
2. to analyse correlation between routine (aPTT) and specialized (factors VIII, IX, XI, XII activity) coagulation tests;
3. to analyse the prevalence of coagulation changes and their differences in different age and gender groups.
1. LITERATURE REVIEW

1.1 Concept of hemostasis

Hemostasis is the physiological process that stops bleeding at the site of an injury while maintaining normal blood flow everywhere else in the circulation [4]. When damage to blood vessel occurs, both from internal or external causes, the bleeding is stopped by the formation of a fibrin clot. When the endothelium of blood vessels are intact it has anticoagulant properties but when injury occurs it leads to the exposure of sub-endothelium to blood plasma. Components in the sub-endothelial matrix are involved in activating the two major processes of hemostasis and the formation of the blood clot. The two main components are known as primary hemostasis, which involves platelets, and secondary hemostasis, which involves the activation of the coagulation cascade. The clot is formed, localized only to the injured endothelium, while the surrounding intact endothelium helps to maintain normal blood flow. The process is initiated and terminated rapidly and is under strict regulation [4-8].

1.2 History of coagulation and its understanding

The study of blood coagulation can be traced back to about 400BC and the father of medicine, Hippocrates [9]. Hippocrates and Aristotle both described in detail various internal and superficial bleeding tendencies. For a long time it was believed that coagulation of blood had to do with cold temperature. It was thought that hemorrhage would stop if the wound was left in contact with the cooling air [8].

The full understanding of hemostasis cannot be attributed to one research alone. It is the work of several different researchers for a long period of time [8, 9]. The major breakthrough occurred during the 20th century, when Paul Morawitz (Morawitz, 1958) described the classic theory of coagulation. He made up a scheme of coagulation that involved four coagulation factors (Figure 1).

It proposed that in the presence of calcium and thromboplastin, prothrombin is converted into thrombin. Thrombin then converts fibrinogen into fibrin, which forms the fibrin clot. He also argued that these factors were present in the blood and the reason the blood did not normally clot was because it was lacking a wettable surface in the blood vessels. This classic theory was used for the next 40 years.

![Fig. 1. Scheme of fibrin generation [8].](image)
In the 1940s, Paul Owren proposed the modern understanding of the coagulation process [8]. He had a bleeding patient in which he could not attribute the pathology to the four coagulation factors, he then discovered the presence of a fifth clotting factor. During the following 10 years several more clotting factors were discovered and they were given roman numerals. The numeric system was assigned to the factors in the order that they were discovered and not according to their role in the coagulation cascade [8-10].

In 1964, two articles, from two independent groups of biochemist, were published introducing coagulation as a step-wise fashion in which activation of one clotting factor leads to the activation of the next. The cascade model by Macfarlane (1964) and the waterfall model by Davie and Ratnoff (1964). These models led to the current understanding of the coagulation cascade (Figure. 2) were the clotting sequence is divided into two pathways, the intrinsic and the extrinsic pathways that both lead to fibrin clot formation via a common pathway [6, 8]. In the 1900th century, German pathologist Rudolph Virchow described thrombi and their tendency to embolize [8]. He proposed that the formation of thrombi is predisposed by abnormalities in blood flow, vessel wall and blood components [11]. This lead to the formation of ‘Virchows’ triad, demonstrated in Figure. 3, for thrombogenesis. It was a simplified view of thrombosis formation predisposition. Now it is known that the process of thrombus formation requires complex interactions involving injury to the vascular endothelium, platelet adherence, aggregation and release and clotting factor activation, eventually leading to thrombin generation and fibrin formation [12].

Fig. 2. The coagulation cascade model [8].
1.3  Physiology of normal hemostasis

The hemostatic process can be divided into 2 major parts: the procoagulant part and the anticoagulant part.

It can also be further subdivided into 3 phases:

- Aggregation and formation of platelet plug – primary hemostasis,
- Clot formation by coagulation cascade – secondary hemostasis,/ Termination of clot formation by antithrombotic control mechanisms,
- Clot removal by fibrinolysis [5].

1.3.1  Primary hemostasis

The first part of the hemostatic process involves platelets, which are anuclear cellular fragments that are derived from megakaryocytes. Platelets become activated when they come into contact with subendothelial matrix, which occurs when a blood vessel is damaged. The response of the platelets is be divided into three phases (Figure. 4). First occurs adhesion of the platelets to the subendothelium of the injured area. Then the platelets start to aggregate, they start attaching to each other making the clot larger. Platelets contain two major intracellular granules containing components involved in further steps. After they have aggregated they start secreting these components with different functions, one of which is recruiting more platelets to the site of injury. The last step of primary hemostasis is the procoagulant activity of the platelets, which includes gathering of enzymes complexes in the coagulation cascade [4, 6-8].
1.3.2 Secondary hemostasis

The secondary hemostasis involves a number of clotting factors and the step-wise activation of them (Figure 2). Since this work is about the deficiency of some of these coagulation factors it is important to explain the function of them in more detail thereby increasing the understanding of the problem.

The majority of coagulation factors were discovered in the middle of the 20th century when doctors and scientists were presented with patients with different coagulation pathologies [4, 6, 9]. While most factors are also named after either the person who discovered them or the patient in whom it was first discovered they have also been given roman numerals. As it was also mentioned previously these numbers were given according to time of discovery and not according to their turn in the coagulation pathway [8].

Coagulation factors are inactivated enzymes or precursor proteins (also called zymogens) that circulate in the blood and become activated when a blood vessel is injured. The activation results in a step-wise amplification of the response. [5].

This step-wise activation of coagulation factors is called the coagulation cascade. The coagulation cascade consists of intrinsic pathway and extrinsic pathway, these two pathways join together in the common pathway, seen in Figure 2 [5, 6, 8].

The first part of the coagulation cascade is differentiated by how it is initiated and the factors involved. The intrinsic pathway is initiated by factors present in the blood while the extrinsic pathway...
is initiated when its components come into contact with tissue factor (TF) located outside the vascular system [5, 8].

The extrinsic pathway is initiated when FVII comes into contact with TF. TF becomes exposed to blood components during injury to endothelium. It activates FVII to FVIIa. TF together with FVIIa then activates FX to its active form FXa. The intrinsic pathway works similarly to the extrinsic pathway. It is initiated when the plasma protein known as Hageman factor (FXII) comes into contact with a negatively charged surface, eg activated platelets. FXII becomes FXIIa. FXIIa together with high molecular weight kininogen (HMWK) activates FXI to FXIa. FXIa, together with FXa and thrombin cleave FVIII to FVIIIa, which acts as a cofactor in the next step. FIXa and FVIIIa, together with calcium and negatively charged phospholipids convert FX to FXa [5, 6, 8].

FXa arises from both the intrinsic and the extrinsic pathway, it is of no importance from where it is activated, the coagulation cascade will continue along the common pathway [8]. The common pathway begins with FXa binding to cofactor FV, and in the presence of calcium this complex forms the prothrombinase complex. This complex converts prothrombin into thrombin, which in turn converts fibrinogen to fibrin and generates the fibrin clot.

The last phase of hemostasis is fibrinolysis. This involves the dissolving of the blood clots during the process of wound healing. It also prevents the formation of clots in healthy blood vessels. The fibrinolytic system is initiated simultaneous with the activation of the coagulation cascade to limit the size of the clot. It is an enzymatic process where plasmin dissolves the fibrin clot into fibrin degradation products. Plasmin, generated from plasminogen by the proteases tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA). Plasmin activity is in turn regulated by its inhibitor, alpha-2-antiplasmin, to prevent widespread fibrinolysis [5, 8].

1.4 Coagulation disorders related to intrinsic coagulation pathway disorders

Disorders of coagulation can involve any part of the hemostasis process leading to hyper- or hypocoagulation. It can be caused by platelet disorders, coagulation factor disorders or fibrinolysis disorders. In this work I have focused on disorders of the intrinsic coagulation pathway, particularly disorders of FVIII, FIX, FXI and FXII.

The definition of coagulopathies can be divided into two different approaches, where one definition involves all types of bleeding disorders, both hyper- and hypocoagulation and the other one involves only disorders of hypocoagulation. It may be defined as follows: Coagulopathy is a pathological condition that reduces the ability of the blood to coagulate resulting in uncontrolled bleeding [14]. This includes the condition when a person has factor deficiencies. There are 13 different
factors in the blood plasma, deficiency of these may cause disturbance of secondary hemostasis and lead to a bleeding disorder. Another definition says that coagulopathy is a disorder in which blood is either too slow or too quick to coagulate (clot) [15]. This definition involves all types of bleeding disorders including thrombocytopenia and/or coagulation factors deficiencies where patients have a prolonged bleeding due to decreased number of platelets and/or decreased activation of coagulation factors as well as thrombophilia where patients have increased risk of blood clot formations due to increased number of platelets and/or increased activation of coagulation factors.

This work is focused on the coagulopathy related to prolonged clotting time usually resulting bleeding disorder.

**Intrinsic coagulation pathway disorders** occur when a patient has a congenital or acquired deficiency in a factor involved in this pathway (prekallikrein, HMWK, FVIII, FIX, FXI and FXII). All pro coagulants are synthesized in the liver, except von Willebrand factor, which is synthesized in megakaryocytes and endothelial cells, and endothelial cells also synthesize FVIII. Due to this, a patient with severe liver failure may acquire an intrinsic coagulation disorder. Half-life of coagulation factors differs significantly this is shown in table 1. As mentioned above, bleeding disorders can be classified into; congenital or acquired and factor deficiencies or acquired inhibitors [5]. Clinical symptoms differ between coagulation factor deficiencies and patients, even between patients with the same disorder, this causes a big dilemma when it comes to the diagnosis and management of such patients [16]. There are 3 grades of coagulation disorders according to the clinical bleeding severity, which can be seen in table 2.

**Table 1. Half-life of intrinsic coagulation factors [17].**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Half-life (h)</th>
<th>Half-life * (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVIII</td>
<td>12</td>
<td>8-12</td>
</tr>
<tr>
<td>FIX</td>
<td>24</td>
<td>18-24</td>
</tr>
<tr>
<td>FXI</td>
<td>80</td>
<td>40-70</td>
</tr>
<tr>
<td>FXII</td>
<td>-</td>
<td>60</td>
</tr>
</tbody>
</table>

* - According Konkle BA [16].
Table 2. Categories of clinical bleeding severity [18].

<table>
<thead>
<tr>
<th>Clinical bleeding severity</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic</td>
<td>No documented bleeding episodes</td>
</tr>
<tr>
<td>Grade I bleeding</td>
<td>Bleeding that occurred after trauma or drug ingestion</td>
</tr>
<tr>
<td>Grade II bleeding</td>
<td>Spontaneous minor bleeding: bruising, ecchymosis, minor wounds, oral cavity bleeding, epitaxis and menorrhagia</td>
</tr>
<tr>
<td>Grade III bleeding</td>
<td>Spontaneous major bleeding: hematomas, hemarthrosis, central nervous system, gastrointestinal and umbilical cord bleeding.</td>
</tr>
</tbody>
</table>

Prevalence and epidemiology of intrinsic coagulation disorders

“1% of the world population is estimated to have a congenital bleeding disorder. Congenital hemophilia A & B, von Willebrand’s disease and inherited qualitative platelet defects make up the majority of these disorders, and the rest is distributed among the rare bleeding disorders“[19]. “A bleeding history is subjective and common symptoms are found in up to 25% of a healthy population without bleeding disorders including epistaxis, gum bleeding, and post-partum haemorrhage” [20]. It is thought that prevalence of coagulation disorders is about 30% of general population and the most of disorders are acquired, but not congenital. It is not possible to know the exact prevalence due to undiagnosed cases.

1.4.1 Factor VIII deficiency

FVIII deficiency, better known as Hemophilia A, is one of the most common types of factor deficiencies. See the compared prevalence of factor deficiencies in table 3.

Table 3. Prevalence and inheritance of intrinsic coagulation disorders [22].

<table>
<thead>
<tr>
<th>Bleeding disorder</th>
<th>Prevalence</th>
<th>Inheritance pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor VIII deficiency</td>
<td>1 in 5 000</td>
<td>X-linked recessive</td>
</tr>
<tr>
<td>Factor IX deficiency</td>
<td>1 in 30 000</td>
<td>X-linked recessive</td>
</tr>
<tr>
<td>Factor XI deficiency</td>
<td>1 in 1 million</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>Factor XII deficiency</td>
<td>1 in 1 million *</td>
<td>Autosomal recessive</td>
</tr>
</tbody>
</table>

* - Azaadet MA, et al. [23].

Hemophilia A is an X-linked recessive genetic disorder. Female carriers have a 50% chance of transmitting the disorder in each pregnancy. Since it is an X-linked disorder, all males born with the faulty gene will be affected and will have clinical expression of the disorder, while females who inherit
the gene are only carriers. Affected males will never transfer to their sons, but all of their daughters will be carriers. There are different degrees of severity ranging from mild, moderate and severe depending in vitro clotting activity. The normal range for clotting activity is approximately 50–150% [21, 22].

The severity of symptoms and the age of diagnosis are usually related to the severity of factor deficiency. This is due to the time of onset and severity of symptoms as seen in table 4.

1.4.2 Factor IX deficiency

FIX deficiency is also known as Hemophilia B. Prevalence of FIX deficiency is reported in table 3. Similar to hemophilia A, this deficiency is also an X-linked recessive genetic disorder transmitted from carrier mother, causing all sons with the same disorder to be affected and all females to be carriers [16, 21, 22].

It is not possible to clinically distinguish the two types of hemophilia from each other. The differentiation between the two needs to be confirmed with laboratory findings of FIX <35 %. The severity of disorder, symptoms and age of usual diagnosis is the same as for hemophilia A, see table 4 [22].

<table>
<thead>
<tr>
<th>Severity</th>
<th>FVIII clotting activity</th>
<th>Symptoms</th>
<th>Usual age of diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe</td>
<td>&lt;1%</td>
<td>Frequent spontaneous bleeding; abnormal bleeding after minor injuries, surgery, or tooth extractions</td>
<td>Age ≤2 years</td>
</tr>
<tr>
<td>Moderately severe</td>
<td>1-5%</td>
<td>Spontaneous bleeding is rare; abnormal bleeding after minor injuries, surgery, or tooth extractions</td>
<td>Age &lt;5-6 years</td>
</tr>
<tr>
<td>Mild</td>
<td>&gt;5-40%</td>
<td>No spontaneous bleeding; abnormal bleeding after major injuries, surgery, or tooth extractions</td>
<td>Often later in life, depending on hemostatic challenges</td>
</tr>
</tbody>
</table>
1.4.3 Factor XI deficiency

FXI deficiency is also known as hemophilia C and it differs from hemophilia A and B in that it affects both males and females. It is an autosomal disorder, and as mentioned it is transmitted to both males and females causing equal clinical expression [16, 21]. The prevalence of FXI deficiency in the general population is written in table 3. However, it was found that this disorder is especially prevalent in a specific ethnic group, Ashkenazi jews. Dr. Rosenthal made this discovery in 1953 after examining two sisters with abnormal bleeding after tooth extractions.

It usually causes a mild bleeding disorder, mostly related to external provocations such as surgery or trauma. Spontaneous bleeding in neonates has not been reported. Women with this disorder are prone to menorrhagia and bleeding during pregnancies [23-25].

1.4.4 Factor XII deficiency

FXII is also known as Hageman factor, it is an enzyme initiating the coagulation factor. It has two major roles in the coagulation cascade one of which is the initiation of fibrinolysis. Due to this, deficiency of FXII does not increase the risk of bleeding like the other factor deficiencies; instead it leads to an increased risk of potential thrombotic events [23].

FXII deficiency is inherited as an autosomal recessive disorder. The prevalence of FXII deficiency is written in table 3, it one of the rarest type of deficiencies. It almost never causes any clinical symptoms and is mainly diagnosed accidentally during a routine coagulation test [24, 26].

1.5 Medicament treatment

The medical treatment of intrinisic coagulation disorders can be difficult. Mainstay therapy is to replace the deficient coagulation factor, this requires the proper investigations to know what factor needs to replaced In some cases specific replacement therapy is unavailable. When the factor deficiency is acquired replacement treatment may be ineffective due to acquired inhibitors, antibodies against the clotting factor concentrate [3, 18].

Most patients with coagulation disorders are only treated when bleeding occurs. In a few cases with severe bleeding of specific deficiencies prophylactic treatment is indicated. Acute bleedings should be treated as soon as possible with administration of appropriate clotting factor. The dosage depend on the required minimal hemostatic level of each coagulation factor and its plasma half-life [5].
When the specific concentrate is not available, plasma is used to correct the deficiency in patients. Fresh frozen plasma (FFP) contains normal levels of stable clotting factors and can be infused in patients with active bleeding [27].

1.6 Screening tests (PT and aPTT) for hemostasis evaluation

As has been mentioned earlier, many of these intrinsic coagulation disorders are asymptomatic and may cause clinical manifestations only during/after surgery or trauma. It is therefore necessary to confirm the diagnosis of bleeding disorder with laboratory testing when it is suspected clinically. Prothrombin (PT) and activated partial thromboplastin time (aPTT) are commonly used tests to detect any abnormalities in coagulation. While they do not specify the exact deficiency, they can indicate the part of the coagulation cascade that is not working as it should [3, 27, 28].

PT is a laboratory test that measures the time necessary to generate fibrin after the activation of FVII. It measures the integrity of the extrinsic coagulation pathway plus the common coagulation pathway, which includes FVII, FV, FX, prothrombin and fibrinogen. This can be measured by recalcifying patient plasma together with an activating agent, tissue factor and phospholipid, and then determining the time it takes to form a fibrin clot. The formation of a fibrin clot can be detect by visual, optical or electromechanical methods. The results varies for each laboratory, normal value usually ranges between 70 – 130 %.

aPTT measures the time it takes to generate fibrin after the initiation of the intrinsic coagulation pathway and the common coagulation pathway. The result is abnormal in case of reduced number of any of the following, FV, FX, FVIII, FIX, FXI, FXII, prothrombin and fibrinogen, the components involved in the intrinsic and common pathways. The aPTT test is performed by recalcifying patient citrated plasma in the presence of a thromboplastic material that does not have tissue factor activity and a negatively charged substances such as kaolin. This results in contact factor activation and the initiation of the intrinsic pathway. The normal aPTT varies in different laboratories, normal value is usually between 25 – 35 seconds [29, 30].

A prolongation of PT and/or aPTT values indicates that there is an abnormal activity in extrinsic and/or intrinsic part of the coagulation pathway respectively. In case of an extrinsic pathway abnormality the PT value will be prolonged since there will be a prolongation of fibrin formation due to some factor deficiency. Similarly, if there is an abnormality in the intrinsic pathway the aPTT value, which measures the integrity of the intrinsic and common pathways, will be prolonged. If both PT and aPTT values are prolonged it can indicate that there is a deficiency in the common pathway, this is explained in a simplified figure of the coagulation cascade (Figure. 5) [3, 7, 18, 29, 30].
Abnormal screening coagulation tests are followed by so called mixing studies to exclude the presence of factor inhibitor. After this, specific factor assays are performed to find the deficient factor. This is important to give correct diagnosis and thereby correct treatment for each patient [18].

In the study Diagnostic outcome of preoperative coagulation testing in children by Bahsin N. et al. conclude that; "routine preoperative coagulation testing identifies only a small number of children at increased risk for surgical bleeding. The clinical utility of such testing must be weighed against the risk to the patient of not identifying a hemostatic defect preoperatively.” [31]. According to this study the usefulness of aPTT/PT testing is limited and it should be used mainly as an adjunctive test to history of patient to make the diagnosis easier.

![Simplified schematic of coagulation cascade indicating laboratory abnormalities depending on type of factor deficiency](image)

**Fig. 5.** Simplified schematic of coagulation cascade indicating laboratory abnormalities depending on type of factor deficiency [3].
2. RESEARCH METHODOLOGY AND METHODS

Study was done at the Lithuanian University of Health Sciences, Kauno Klinikos at the Department of Laboratory Medicine.

2.1 Study population

Patient criteria for this study included patients that had been in departments of Lithuanian University of Health Sciences, Kauno Klinikos, and patients that had been consulted in outpatient department from 1st of January to 31st of December 2016. Analysis of demographic, clinical and laboratory data was done. In this study was used data collected from patients were screening tests – SPA and aPTT had been done. From this group of patients 1 or more specific coagulation factors of the intrinsic coagulation system (FVIII, FIX, FXI and FXII) were tested Coagulation factors analyses were done and the correlation between factor activities and screening tests results was analysed.

Further in the study the patients with isolated abnormal SPA were excluded from the study to leave only patients with abnormal aPTT, which reflects deficiencies of intrinsic coagulation factors.

The data from adults and children more than 1 year was used in this study.

In the study the patients were divided into groups according to the indications for coagulation testing:

- patients with abnormal screening coagulation test findings who were advised to seek consultation from laboratory medicine specialist;
- patients with active bleeding or previously known history of intrinsic coagulation disorder, mostly patients on follow-up for treatment;
- patients without active bleeding, but with suspected (unclear) personal or family history of bleeding.

2.2 Research methods

2.2.1 Blood sampling

The same method was used for all coagulation tests. Venous blood was collected according to standard operating procedures (WHO guidelines on drawing blood) [32].

The tube used contained 0.109 M of buffered trisodium citrate solution (BD Vacutainer, USA). This was used as an anticoagulant for coagulation investigations. Its anticoagulant effect is by binding to metal ions such as calcium, which inhibits the coagulation cascade. The citrate solution in
the BD vacutube acts as a buffer, so no additional buffers were added as they can adversely affect laboratory studies.

It was important to get the correct ratio of blood to additive in the tubes; this was carefully controlled during the procedure. The ratio was 9 volumes of blood to 1 volume of trisodium citrate anticoagulant. All BD Vacutainer coagulation tubes were marked indicating the minimum fill level of blood to additive and then filled with the correct amount of blood.

A site for venipuncture on the patient was chosen; most commonly the antecubital area was used. A tourniquet was then applied above the selected venipuncture site and the patient was asked to clench his/her fist to make the vein more accessible. The person performing the puncture put on gloves and disinfected the site. A hypodermic needle was then used to puncture the vein and the vacutube was applied to the other end of the needle for collecting the blood. When the desired amount of blood had been collected the performer removed the tourniquet and then the needle (WHO guidelines on drawing blood) [32].

2.2.2 Specimen preparation

Plasmas were separated via centrifugation for 15 minutes at 2500×g, speed with centrifuge model Eppendorf 5810 (Eppendorf, Germany). The same method was used for all plasma samples.

Sample storage

Plasmas were stored according to coagulation test they were to be used for. Samples intended for Stago Prothrombin Assay (SPA) and aPTT testing were utilized without delay. Samples for testing the activity of coagulation FVIII, FIX, FXI or FXII were frozen at -20°C degrees and where they were kept for no longer than 15 days. The freezing and thawing procedure of these samples was not repeated; once a sampled had been thawed it could not be frozen again. Therefore the samples were divided into different tubes to be able to perform activity test of different factors if needed without having to repeat the blood drawing procedure.

Test methodology; the test methodology for the activity of different factors are similar, but the measurement of SPA and aPTT differs from the other tests.
2.2.3 Measurement of Protrombin Time

**Reagents**

STA-SPA+ reagent kit (Diagnostica Stago, France) was used for determination of the combined factors II-VII-X.

**Equipment**

Coagulation analyser Compact Max (Diagnostica Stago, France) was used in the investigation of coagulation time. The same equipment was used for all the coagulation tests.

**Test principle**

The principle consists of the measurement of the clotting time, in the presence of tissue thromboplastin, of a system in which all the factors were present and in excess (supplied by the STA – SPA +) except FII, FVII and FX which were derived from the plasma being tested.

**Procedure**

Calibration was done according manufacturers recommendations. For quality control STA – ScandiNorm (REF 00106) was used for normal level and STA – ScandiPath (REF 00107) was used for abnormal level. Two levels of measurement were done. Steps of calibration and control measurements were done under the manufacturer recommendations reported in the reference manual.

The patients’ plasma was tested undiluted; it was loaded into the instrument in accordance to the instruction manual for the analyzer model. Steps of analysis were carried out under the manufacturer recommendations for sample testing reported in the Coagulation analyser Compact Max reference manual.

**Results evaluation and interpretation**

Normal reference interval of SPA is 70–130 %. Any increase or decrease of this value indicates pathology of the extrinsic coagulation pathway.

Limitations of usefulness of this test may occur if samples have not been handled correctly, the slightest coagulation (micro-clots) will lead to shortening of times measured; while extensive coagulation will prolong the clotting times due to consumption of factors and fibrinogen. The test may also be prolonged by high doses of heparin and the rare presence of anti-bovine factor V antibodies.

2.2.4 Measurement of Activated Partial Tromboplastin Time (aPTT)

**Reagents**

STA-PTT AUTOMATE reagent kit (Diagnostica Stago, France) was used for determination of activated partial thromboplastin time.
**Equipment**

The equipment used is described in 2.2.3.

**Test principle**

The re-calcification time of plasma in the presence of cephalin (a thrombocyte substitute) and particle activator (silicone) of non-settling (standardized activation of FXII) was measured.

Thereby examining the integrity of the intrinsic and common coagulation pathway (FXII, FXI, FIX, FVIII, FX, FV, FII and FII) with the exception of platelets.

**Procedure**

Calibration of test is described in 2.2.3. Quality control Coag Control N+P (REF 00621) were used for internal quality control.

**Results evaluation and interpretation**

Normal reference interval is 28 – 38 seconds. Prolongation of aPTT is related to pathology that could be caused by intrinsic factor deficiencies (FXII, FXI, FIX, FVIII, FX, FV, FII), use of heparin or the presence of anticoagulants.

**Limitation of use**

Limitations of use are mostly related to monitoring of heparin treatment, no special limitations for aPTT use in factor deficiencies evaluation.

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**2.2.5 Determining activity of factor VIII (FVIII)**

**Reagents**

STA-DEFICIENT VIII reagent kit (Diagnostica Stago, France) with Immuno-depleted plasma for FVIII assay was used for determination of FVIII activity.

**Equipment**

The equipment used is described in 2.2.3.

**Test principle**

The assay consists of the measurement of the clotting time, in the presence of cephalin and activator, of a system in which all the factors are present and in excess (supplied by STA- Deficient VIII) except FVIII which is derived from the sample being tested (1, 3).

**Procedure**

Calibration of test is described in 2.2.3. It is necessary to run controls in order to ensure accuracy and reproducibility of the results. Two different levels of controls were used. The STA-System control N+P was used for quality control. The same method of quality control and procedure of determining activity of factors was used for all factor deficiency tests.
Steps of analysis were done under the recommendations reported in the reference manual. The analyzer automatically carried out FVIII assays of the plasmas that were tested.

**Results evaluation and interpretation**

The normal plasma range of FVIII used in this study between 60–150%. Any value outside the reference range, increased or decreased, is indicative of a coagulation disorder in the patient. There could be a congenital or acquired cause of this pathology. A decreased value of FVIII is known as hemophilia A.

Presence of heparins and thrombin inhibitors in the plasma tested may lead to an underestimation of the factor VIII level, as well as the presence of lupus anticoagulants in plasma. This may lead to limitations of the tests usefulness.

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**2.2.6 Determining activity of factor IX (FIX)**

**Reagents**

STA-DEFICIENT IX (Diagnostica Stago, France) Immuno-depleted plasma for factor IX assay was used for determination of FIX activity.

**Equipment**

The equipment used is described in 2.2.3.

**Test principle**

The assay consists of the measurement of the clotting time, in the presence of cephalin and activator, of a system in which all the factors are present and in excess (supplied by STA-Deficient IX) except FIX which is derived from the sample being tested.

**Procedure**

The procedure of determining activity of FIX was the same as for other factor activity tests and it is described in 2.2.5.

**Results evaluation and interpretation of FIX results**

The normal plasma range of FIX used in this study is between 60-150%. Any increase or decrease in this value indicates intrinsic coagulation disorder. This can be caused by congenital or acquired pathology. A decreased value for FIX is called Hemophilia B.

Limitation of use of test is described in 2.2.5.
2.2.7 Determining activity of factor XI (FXI)

**Reagents**
STA-DEFICIENT XI (Diagnostica Stago, France) Deficient plasma for factor XI was used for determination of FXI activity.

**Equipment**
Equipment used is described in 2.2.3.

**Test principle**
The assay consists of the measurement of the clotting time, in the presence of cephalin and activator, of a system in which all the factors are present and in excess (supplied by STA-Deficient XI) except FXI which is derived from the sample being tested.

**Procedure**
The procedure of determining activity of FXI was the same as for other factor activity tests and it is described in 2.2.5.

**Results evaluation and interpretation**
The normal plasma range of FXI used in this study is between 60-140%. In the newborn, the factor XI level is low (30 – 50% of adult values).

Any increase or decrease in the value is indicative of intrinsic coagulation disorder. A decreased value of FXI is called Hemophilia C.

Limitation of use of test is described in 2.2.5.

2.2.8 Determining activity of factor XII (FXII)

**Reagents**
STA-DEFICIENT XII (Diagnostica Stago, France) Deficient plasma for factor XII was used for determination of FXII activity.

**Equipment**
Equipment used is described in 2.2.3.

**Test principle**
The assay consists of the measurement of the clotting time, in the presence of cephalin and activator, of a system in which all the factors are present and in excess (supplied by STA-Deficient XII) except FXII which is derived from the sample being tested.

**Procedure**
The procedure of determining activity of FXII was the same as for other factor activity tests and it is described in 2.2.5.
Results evaluation and interpretation

The normal plasma range of FXII used in this study is between 60-140%. Decreased or increased value is indicative of intrinsic coagulation disorder. A decreased value of FXII, unlike decreased value of FVIII, FIX and FXI, does not lead to an increased tendency of bleeding.

Limitation of use of test is described in 2.2.5.

2.3 Statistical analysis

Statistical Software IBM SPSS Statistics v23.0 (IBM, USA) was used for data statistical evaluation. The Mann-Whitney $U$ test was used to assess the statistical significance of differences between the groups. Correlations were estimated by the Spearman’s rank correlation test. Qualitative data analysis for independence and relationships of variables was performed using chi-square test. The Fisher’s exact test was used for small sample sizes. Statistical significance was assumed at $P<0.05$. 
3. RESULTS

In this study there were included 99 patients in total. 49 were male and 50 were female. Using nonparametric test with independent samples it was observed that there was no difference in distribution of age between male and female included in the study. Mean age of male was $22.9 \pm 23.1$ and the mean age of females was $32.3 \pm 27.59$ ($p=0.95$).

*Clinical symptoms (indications of testing) and aPTT abnormality*

Patients groups according clinical symptomatic and indications for testing were grouped into 3 groups: absence of bleeding, but pathological screening coagulation results – 32 (32.3%), active bleeding symptoms – 20 (20.2%), history of bleeding – 47 (47.5%). Prevalence of clinical symptoms or indications type for testing did not differ significantly in man and women ($Chi-square = 2.833$, $df=2$, $n=99$, $P=0.264$) (Figure. 6).

During coagulation screening 70.7% of patients were with prolonged aPTT results. Relations between aPTT results and clinical symptomatic/indications for testing was analyzed. Significant difference in proportion of pathological aPTT in different patients groups (by clinical symptomatic/indications) was found ($Chi-square = 17.341$, $df=2$, $n=99$, $P=0.000$).

![Fig. 6. Correlation between aPTT changes and clinical data.](image-url)
**Prevalence of aPTT abnormality in relation to gender**

Chi-square test was used to determine whether the prevalence of aPTT abnormality is related to gender. It was determined that there is no significant difference in aPTT prolongation prevalence between male and female gender (Chi-square = 1.081, df=1, n=99, P=0.378) (Figure 7).

![Figure 7. Distribution of aPTT results according to patients gender](image)

**Prevalence of aPTT abnormality in relation to gender and patients age**

In order to analyze the prevalence of aPTT abnormality in different ages the 99 patients that were used in this study were divided into two age groups:

1. **Children** – those that were ≤18 years old and
2. **Adults** – those that were >18 years old.

61 patients and 38 patients were in each group, respectively. The frequency of pathological aPTT results did not differ significantly between children and adults (Chi-square= 0.937, df=1, n=99, P=0.372) (Figure 8).
In order to analyze possible differences of disorders prevalence in relation to gender in different age groups children and adults were analyzed separately. Children group consisted of 33 male (54.1%) and 29 female (45.9%). It was found that there is no significant difference in prevalence of aPTT abnormality in male or female patients ≤18 years (Chi-square = 0.201, df=1, n=61, P=0.786).

To determine the prevalence of aPTT abnormality by gender in adult patients Fisher exact test was used. The group consisted of 38 patients: male – 16 (42.1%), female – 22 (57.9%). It was found that there is no significant difference in prevalence of aPTT abnormality in male or female adult patients (Fisher = no coefficient values, P=0.254).

70 patients with increased aPTT were analyzed in order to detect coagulation factors FVIII, FIX, FXI and FXII deficiencies. Coagulation factors deficiencies were detected in 38 of 70 patients (54.4%) with increased aPTT. Relation between prevalence of aPTT changes and prevalence of intrinsic factors changes was significant (Chi-square = 17.124, df=1, n=99, P=0.000).

**FVIII activity analysis: correlation with aPTT and prevalence of FVIII changes**

93 out of the 99 patients in this study were tested for FVIII activity. Spearman's correlation was used to calculate the relationship between aPTT and F8 activity, the results show that there was a significant correlation between aPTT values and FVIII activity (r=-0.242, P=0.019) (Figure. 9). It can be seen in the results that the amount of FVIII deficiency is negatively correlated to the prolongation of aPTT. It could be believed that the bigger the deficiency of FVIII the longer aPTT should be, according to the results this was proved.
47 (50%) patients had a normal activity of FVIII, 25 (27%) of these had a decreased activity of FVIII and 21 (23%) patients had an increased activity of FVIII. In 24 out of the 25 patients with decreased activity of FVIII the aPTT was prolonged, but in one patient aPTT stayed within normal limits. Analysing 21 patients with increased FVIII activity was observed that 10 of them had an aPTT within normal limit, but 11 had prolonged aPTT (in these cases is recommended to look for other intrinsic factor deficiencies). aPTT is not so sensitive for evaluation of the increased activity of FVIII. 34 patients with normal activity of FVIII had prolonged aPTT, this could be because some of the patients could have deficiency of some other clotting factor.

**FIX activity analysis: correlation with aPTT and prevalence of FIX changes**

47 out of the 99 patients in this study were tested for FIX activity. Spearman's was used to calculate the correlation of aPTT prolongation and FIX disorder. The results show that there was no significant correlation between the prolongation of aPTT and FIX disorder \(r=-0.238, P=0.111\) (Figure 10).

38 (81%) patients had a normal FIX activity, 7 (15%) patients had deficient activity and 2 (4%) patients had increased values. All patients with abnormal factor activity had a prolonged aPTT. 33 patients with normal FIX activity had prolongation of aPTT (other reasons for prolongation), only 5 patients had aPTT within normal range. Only three patients had been tested only for FIX, two of them had normal values of both FIX and aPTT and one had a decreased activity of factor IX and prolongation of aPTT.
FXI activity analysis: correlation with aPTT and prevalence of FXI changes

32 out of the 99 patients in this study were tested for FXI activity. Spearman’s was used to calculate the correlation of aPTT prolongation and FXI disorder, the results show that there was no significant correlation between the prolongation of aPTT and FXI disorder ($r=-0.129$, $P=0.482$) (Figure 11).

FXI activity was tested in 34 of the 99 patients. Out of these 34 patients 26 (76%) had normal value of FXI activity, four patients (12%) had a decreased value and another four patients (12%) had increased value. Out of the 26 patients with normal FXI activity 25 had prolongation of aPTT (possible other factor deficiencies), the four patients with decreased FXI had prolongation of aPTT while the four patients with increased activity also had a prolongation of aPTT. Similarly like with FVIII activity, aPTT is not sensitive to these types of FXI changes. One patient with normal FXI activity had aPTT within normal limit. One patient had only factor XI activity tested showing a decreased FXI activity and increased aPTT.
FXII activity analysis: correlation with aPTT and prevalence of FXII changes

29 out of the 99 patients in this study were tested for FXII activity. Spearmans was used to calculate the correlation of aPTT prolongation and FXII disorder, the results show that there was a significant correletation between aPTT prolongation and FXII disorder ($r=-0.461$, $P=0.012$) (Figure 12).

30 out of the 99 patients had FXII activity measured. 13 patients (43%) had normal FXII value, 17 patients (57%) were found with decreased activity, no patients with increased factor activity. All patients with decreased value of FXII had prolonged aPTT, 10 patients with normal FXII and increased value of aPTT and 3 patients were within normal ranges in both FXII activity and aPTT.

![Figure 11. Correlation between aPTT and FXI value](image)
**Fig. 12. Correlation between aPTT and FXII value**

*Prevalence of intrinsic factor activities changes in relation to gender and patients age*

To see if there was a difference between the prevalence of intrinsic factor disorders (FVIII, FIX, FXI and FXII) in male and female gender according to age group the analysis was done in the two separate age groups.

In the age group ≤18 years the results were:
- FVIII: (Fisher value=1,727, P=0,450),
- FIX: (Fisher value=*no coefficient values*, P=0,470),
- FXI: (Fisher value=1,194, P=0,774),
- FXII: (Fisher value=*no coefficient values*, P=1,000)

The results show that there is no significant difference in the prevalence of disorders of all four intrinsic coagulation factors in relation to male and female gender in patients ≤18 years.

In the age group >18 years the results were:
- FVIII: (Fisher value=1,247, P=0,623),
- FIX: (Fisher value=1,478, P=0,743),
- FXI: the difference in prevalence of F11 disorders between male and female gender could not be evaluated as all patients with F11 disorder in this study were female gender,
- FXII: (Fisher value=*no coefficient values*, P=1,000)

The results show that there is no significant difference in the prevalence of disorders of all four intrinsic coagulation factors in relation to male and female gender in patients >18 years.
4. DISCUSSION OF RESULTS

Bleeding is a common symptom and does not always indicate an underlying bleeding disorder [3]. Identification of true coagulation disorders may prove challenging. The relative frequency and symptoms of pathologic and nonpathological bleeding are similar. Evaluation of patients is based on obtaining thorough personal and family histories and laboratory evaluation of a complete blood count and screening coagulation studies. The results of screening coagulation studies can be used to narrow down the possible disorders.

Our study was done in order to evaluate the contribution of routine and specialized coagulation laboratory testing to clinical decision making in suspected intrinsic coagulation disorders. The sensitivity of aPTT may differ to different coagulation factors, this may lead to different results regarding the correlations between aPTT prolongation and factor deficiencies. In our study we aimed to assess correlation between aPTT and different factor disorders.

During screening coagulation studies we found 70.7% of patients with isolated aPTT prolongation. We found significant difference in proportion of pathological aPTT in patients groups with different clinical symptomatic/indications for coagulation testing. Coagulation factors deficiencies were detected in more than half patients with increased aPTT. Isolated prolongation of the aPTT indicates an abnormality of the intrinsic pathway (FVIII, FIX, FXI, FXII deficiencies), but it can also indicate a lupus anticoagulant or small doses of heparin treatment or heparin contamination in test sample. In our study presence of lupus anticoagulant and/or heparin was controled – patients with these conditions were not included in the study.

We observed that in patients with intrinsic coagulation pathway disorders overall aPTT sensitivity was near to 100%. Theoretically, when there is a factor deficiency there should be an aPTT prolongation. Since a clotting factor deficiency causes problems in the clotting system it takes longer for the blood to form clots. Our findings allowed us to prove this. There is some controversy regarding the differential utility and sensitivity aPTT assay methods [33].

The calculations of factor activity and aPTT correlation in this study concluded that FVIII and FXII activity showed correlation with aPTT, while FIX and FXI activity showed no correlation with aPTT.

Some patients had only single clotting factors tested while others had two or more tests. FXI only one patient with normal factor activity had normal aPTT while the 25 other patients with normal factor activity had a prolonged aPTT. This does not mean that the aPTT value for the 25 patients with normal FXI activity was incorrect, it could be that these patients had other clotting factor deficiencies causing the prolongation of aPTT or that there were other causes of aPTT prolongation – inhibitors to
clotting factors or others [3, 33]. It is known that the most common cause of prolonged aPTT is incorrect collection of the blood sample.

So while there may be many causes for the non-correlation between FIX, FXI and aPTT this study shows that aPTT will give correct correlations in about half of the cases. This questions the usefulness of aPTT as screening test. The value of a screening test must be measured against the risks of not performing any screening test especially before major surgery etc. Bhasin N. and ect. studied the value of routine coagulation testing instead of bleeding history alone in children to predict the risk of bleeding prior to tonsillectomy and adenoidectomy. They used a larger study group of 792 pediatric patients. They then proceeded to test the value of aPTT in these children vs. bleeding history alone. They concluded, as earlier studies had demonstrated that “routine coagulation testing identifies only a small number of children at increased risk for surgical bleeding” and that “value of such testing must be weighed against the risk to the patient of not identifying a haemostatic defect preoperatively”. They found that approximately 1/3 of the patients had normal PT/aPTT on repeated testing. Bhasin N. and ect. therefore recommended a repeat testing before moving on to more specialized coagulation testing [31]. They also found that a positive personal/family history of bleeding only identified half of the children that were found to have coagulation disorders with the use of specialized clotting tests.

Similarly to that study, our study showed that, while aPTT is not a reliable test to use alone it can be useful as an adjunct to personal/family history. It can be especially useful in those planned to undergo major surgery where risk of bleeding is increased.

Because the results of aPTT in this study differs a lot without any specific guides to follow I believe that in order for this type of study to give more conclusive results the study group should be bigger, since this would yield a more certain result regarding the correlation between aPTT and specialized tests.

There was no significant difference in the distribution of gender in either age group. It is known that Hemophilia A and B are X-linked disorders that pass on from mother to son. This means that the majority of patients, especially in the group of 18 years or less, I would expect there to be a majority of male patients, but there was no significant difference in the distribution of gender in either age group. The results of the study do not reflect theoretical knowledge because of the heterogeneity of study population – patients included in the study varied a lot according their coagulation disorders. High concentration of different pathologies is common in the university hospitals.

As argued in the segment about factor activity and aPTT correlation, using aPTT alone is not enough to confirm or dismiss a diagnosis of bleeding disorder because of unforeseeable results. The value of aPTT as a screening test is that it is one test that can give the clinician an idea of the presence or absence of bleeding disorder, when aPTT measurement is done in combination with PT
measurement (which measures the time of extrinsic and combine coagulation pathway) it indicates to what part of coagulation pathway may be deficient and on what specific factors to focus on.

In this study we see that the degree of aPTT prolongation does not correlate well with the degree of FIX and FXI deficiency. Some patients with severe factor deficiency may have shorter aPTT than patients with only moderate/mild factor deficiency. Our study results don’t stand out compared to data of other researches. It is known that APTT reagents show different sensitivities to deficiencies of factors VIII, IX, XI and XII; this is thought to be because of differences in the activator or phospholipids used in the reagent [34]. Another limitation with aPTT is that it does not specify what specific clotting factor is deficient or other reasons, like lupus anticoagulant or small doses of heparin treatment. Additional mixing studies could be helpful in these cases.

Since diagnostics possibilities and treatment differs depending on severity and specific factor deficiency it is important do specialized laboratory coagulation testing for the clinician to be able to provide the patient with proper diagnosis and adequate treatment. Despite some current controversy regarding the differential utility and sensitivity of various assay methods for aPTT and for coagulation factors activities assays, it is advisable to combine good clinical evaluation and screening tests of coagulation disorders to orient efforts to diagnosing coagulation disorder.
5. CONCLUSION

1. The correlation between aPTT test results with clinical and anamnestic data differs depending on relevance of the data. In patients with clear indications, active bleeding, for screening coagulation tests the correlation with clinical status was strong and significant. With these results we can conclude that in combination with a good clinical evaluation, screening tests of coagulation disorders is a valuable adjunct to diagnosing coagulation disorder.

2. It was determined that the correlation between routine and specialized coagulation tests was depending on coagulation disorder type. There was a significant correlation between aPTT value with the activity of FVIII and FXII, while there was no correlation between aPTT value with the activity of FIX and FXI. aPTT usage for the screening of intrinsic pathway coagulation disorders related to factor deficiencies is reliable.

3. It was found in this study that the overall prevalence of changes in routine and specialized coagulation tests does not differ in the two age groups or between male and female gender. The distribution in prevalence of the different factor disorders does not differ in the patients of both age groups (children, ≤18 years and adults >18 years old).
1. REFERENCES


ANNEX 1

LIETUVOS SVEIKATOS MOKSLŲ UNIVERSITETAS
BIOETIKOS CENTRAS

Medicinos akademijos (MA)  2016-05-02  Nr. BUC-MF-035
Vientsiųų studijų programa – MEDICINA
V k. stud. Amanda Vest

DĖL PRITARIMO TYRIMUI

LSMU Bioetikos centras, įvertinęs (MA) vientsiųų studijų programos – MEDICINA
V k. stud. Amanda Vest (mokslinio darbo vadovė: doc. Daiva Urbonienė, LSMUL KK
Laboratorinės medicinos klinika) mokslinio-tiriamojo darbo temos: „Intrinsic coagulation
pathway disorders and laboratory diagnostics correlations“ tiriamojo darbo anotacija, kuri leidžia
spręsti, jog planuojamame tyrimo neturėtų būti pažeistos tiriamojo teisės, todėl šiam tyrimiui
pritariama.

Bioetikos centro vadovo pavaduotojas  
doc. E. Peičius