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REVIEW ARTICLE



# The biochemical approach to thromboembolism: the relevance of molecular aspects

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## ABSTRACT

**Introduction.** Arterial and venous thromboembolism is a disease with a high impact on morbidity and mortality. Their pathological mechanisms of aggregation directed by the clotting factors along with the variations in clinical manifestation are regarded to a high moiety of genetic polymorphisms along with a wide diversity of comorbidities.

**Material and methods.** A comprehensive literature review was conducted, which included a total of 119 sources. Among these, 60 sources were systematically collected, while the remaining 59 sources were selected through non-systematic methods.

**Results.** We have identified different treatment options that regard both the venous or arterial thromboembolism in contrast with numerous pathogenetic outcomes, population groups along with biomarkers that significantly modify the clinical aspects of the therapeutical and post-clinical treatment aspect. At the moment its diagnosis is continuously improving worldwide, taking into consideration a high diversity of experts' opinions with a wide practical experience.

**Conclusions.** Arterial and venous thromboembolisms are serious medical conditions that can be prevented and effectively managed with modern diagnostic and therapeutic techniques.

**Keywords:** arterial/venous thromboembolism, biomarkers, blood, coagulation.

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## Key messages

### What is not yet known about the issue addressed in the submitted manuscript

Currently, there are no biomarkers available that can guarantee a 100% accuracy in the differential diagnosis of arterial and venous thromboembolism, thereby increasing the complexity of the diagnostic process.

### The research hypothesis

There are different biomarkers which in conjunction with each other can leave a trace of different conditions thus implying the necessity of using these molecular titers in a sensible and specific way. The biomarkers which are most efficient must be identified along with the auxiliary clinical methods for the management of thromboembolism.

### The novelty added by manuscript to the already published scientific literature

Many studies consider the molecules either the surgical methods that are acceptable in daily clinical routine in an isolated manner but none of them have tried to see a broad picture of this memorable pathological condition.

## Introduction

In-depth research reveals thromboembolism as a significant global issue that demands a timely diagnosis for swift assessment and appropriate treatment. The utilization of thrombosis markers in line with the diagnostic algorithm and disease stage remains an undiscovered aspect that warrants exploration [1].

Thromboembolism is a disease, which can affect various anatomical vessels, including arterial, venous and central portion [2-4]. Morphologically it is represented by thrombogenesis that will in turn lead to blood flow obstruction in that anatomical region [5].

Arterial thromboembolism was investigated with less detail. It can arise from the central region of the cardiovascular system or from atherosclerotic plaques in large-diameter blood vessels [4]. In certain animals, this condition can be extremely painful and debilitating, raising ethical concerns in veterinary medicine regarding euthanasia as a means to alleviate suffering [6].

The contemporary concept describes the mechanism of the coagulation cascade in which the factors are designated by Roman numerals, except for the first four factors which have specific names: Factor I (fibrinogen), Factor II (prothrombin), Factor III (tissue factor), and Factor IV (bivalent calcium ions). These factors can interact with each other to form a primary thrombus, which will undergo further fibrinolysis [7].

It has been observed that the main factors that are associated with the arterial thromboembolism are the anomalies in the coagulation cascade, cytokines, the soluble form of the P-selectin, the elevation of clotting factors, thrombocytosis and leukocytosis [4]. These factors are more marked in advanced age, females, and during exacerbation of comorbidities such as arterial hypertension, diabetes mellitus, myocardial infarction, heart failure, and stroke [8].

In the context of the emerging SARS-CoV-2 pandemic and COVID-19, there has been observed an increase in the incidence of thromboembolism and signs of ischemia following heart failure. The highest incidence of arterial thromboembolism has been identified in the lower limbs (71%), upper limbs (14%), mesenteric arteries (4%), and the arterial Willis' circle (10%). In certain cases, multiple sites may be affected, and there is a possibility of concomitant venous thromboembolism [9].

It has been demonstrated that in COVID-19, pulmonary venous thromboembolism is more prevalent than deep venous thromboembolism. This is attributed to the heart lesions that are induced in the cytokine storm that is stimulated by the virus antigens, without a primary focus on venous thromboembolism, contrary to initial reports from Wuhan, Hubei province, China, and surrounding regions, as indicated by a meta-analysis [10].

Malignant neoplasms are characterized by arterial vascularization and subsequent dissemination of thromboemboli in the arterial system. The highest incidence of arterial thromboembolism has been observed in cases of cerebral, pulmonary, colorectal, and pancreatic cancer [11].

The most prominent and up-to-date marker for thrombosis is D-dimer, which can be elevated in the elderly population, as well as in cases of cancer, infections, and chronic inflammations. This biomarker exhibits high sensitivity but limited specificity for venous thromboembolism [2]. Additionally, D-dimer levels can be influenced by various genetic polymorphisms that affect fibrinogen sequences, which may reduce its reliability in a clinical setting [12].

The Zaharin-Head regions have been shown to reflect somatosensory sensations, such as acute or chronic pain, and can serve as markers of organic lesions in the preterminal or terminal stages [13]. In cases of upper limb arterial occlusion, the pain may manifest as sensations of itching and numbness along the ulnar nerve pathway [14].

The objective of this study was to investigate the biochemical mechanisms underlying thromboembolism, with the aim of identifying effective therapeutic approaches and highlighting future research directions in the areas of prevention and treatment. We specifically focused on exploring the interactions between various biomarkers and their potential impact on avoiding quantitative biases in biochemical marker analysis.

## Material and methods

A randomized literature study was conducted on 01.02.2023 in order to identify randomized clinical trials, meta-analyses and review articles. The search was conducted using numerous platforms including the PubMed, HINARI, EMBASE and Elsevier libraries.

After applying a set of criteria, a list of 60 random papers were selected. The including criteria were: relevance of the topic, had at least one of the key-words in the title or abstract – “thrombosis”, “thromboembolism” in combination or with the “arterial”, “venous” or “pulmonary” and were coincident with our era and prevalent lifestyles on a cultural background with a relatively strict selection of the sources from the past 10 years. Some exceptions were admitted for the time period for the literary sources which are still standing as leading. The exclusion criteria were: outdated information, irrelevant geographical location and irrelevant time period of the results.

For an increased confidence of the research query were independently studied 59 sources. There were details that required additional clarification this in turn demanding this search. Overall, we have studied 119 literary pieces. All of them had to be regarded as being of high quality in respect to the journal in which they were published and its impact factor.

We did not follow any current guidelines, but we have used a protocol enlisted in the Essential Evidence-Based Medicine, 2<sup>nd</sup> edition by Dan Mayer [15].

## Results and discussion

### General aspects

Hypercoagulability can be defined as a condition characterized by an increased propensity for blood clot formation, influenced by both endogenous and exogenous factors. It is important to note that arterial hypercoagulability differs

significantly from venous and central hypercoagulability [16]. A notable physiological aspect of hypercoagulability is the Virchow's triad, which associates alterations in local blood circulation with blood vessel lesions [17].

Comorbidities associated with an increased risk of thrombosis are classified as prethrombotic states, while spontaneous thrombosis represents the primary pathology. Notable causes of thrombosis often involve deficiencies in protein S, protein C, antithrombin III, as well as various dysfibrinogenemias. Malignant conditions, pregnancy, oral contraceptives, myeloproliferative disorders, hyperlipidemia, diabetes mellitus, and vascular anomalies, along with alterations in blood rheology, are also considered prethrombotic states [18]. Hemophilic infections and viral infections are the primary contributors to both venous and arterial thromboembolism. These infections can be further complicated after splenectomies or during leukemia, potentially leading to confusion in laboratory data interpretation [19, 20].

It has been demonstrated that geomagnetic storms may have a potential correlation with increased incidence of cardiovascular diseases, including myocardial ischemia and cerebral stroke. The intensity of radiation emitted on the Earth's surface is higher during wintertime due to the planet's proximity to the sun, which contrasts with the equatorial region where there is a higher incidence of cardiovascular diseases throughout the year due to increased radiation exposure [21].

Comorbidities play a significant role in these mechanisms as they can affect both the quantity and quality of clotting factors, thereby modifying their functionality and impacting fibrinolysis. In kidney diseases, a tendency towards hypocoagulability is observed, while in chronic cardiac pathology, states of hypercoagulability are observed. The coagulation index can be measured using an integral approach that assesses the fibrinolytic potential and the overall hemostatic potential [22].

To understand the coagulation system in relation to other systems, it is important to consider a collection of intracellular operons that are based on microparticles. These granules can be observed in conditions such as atherothrombosis or type 2 diabetes mellitus, where they can be activated without a physiological reason and initiate the coagulation cascade involving tissue factors and factor VIIa. This provides an explanation for the occurrence of thromboembolic events in patients with these diseases [23-26].

### **Arterial thromboembolism**

The factor which can determine the arterial thromboembolism is usually the fibrinogen that will in turn be transformed into fibrin (which can lead to the COVID-19 ground-glass pneumonia along with the vitamin K, coagulation factor XIII which is responsible for the fibrin formation from fibrinogen and von Leiden factor (V)) [27]. The main integrins that represent the subunits which constitute the membrane receptors that are implicated in the coagulation cascade are represented by the glycoprotein Ia, glycoprotein Ib, glycoprotein IIb, glycoprotein V and glycoprotein IX [28].

Thrombomodulin activates protein C and thus it represents a mediating factor for the thrombotic states. The platelet surface receptor which will lead to thrombogenesis are surface glycoprotein IIb/IIIa, Ia/IIa and Ib/IX/V [27].

It is well-known that oral contraceptives are a major risk factor for the arterial thrombosis but the mechanism is scarcely mentioned. A rising concentration of clotting factors in the blood circulation determined by these drugs will in turn lead to a primary arterial thromboembolic event [29].

Thrombocytes possess an intracellular microsomal system known as the secretome, which is activated in a cascade manner upon stimulation of the aforementioned receptors. This system involves the secretion of  $\beta$ -thromboglobulins from  $\alpha$ -granules located on the surface of monocytes, granulocytes, T-lymphocytes, and mastocytes. In the context of arterial thromboembolism in the cerebral vascular system, the direct involvement of matrix metalloproteinases MMP-2 and MMP-9 has been observed in the mechanisms associated with the secretome, thus establishing their connection with the proteasome [30].

The platelet secretome has been found to play a significant role in the pathogenesis of osteoarthritis, particularly through the amplified secretion of interleukin 17 and interleukin 17A. This leads to the activation of p38 and p65, which further enhance the expression of the NF- $\kappa$ B pathway. Consequently, matrix metalloproteinases MMP-1, MMP-3, MMP-9, and MMP-13 are activated, contributing to the regulation of anabolic hormone synthesis [31]. Additionally, a correlation was demonstrated between the tissue inhibitor of metalloproteinase-1 (TIMP-1), heat shock protein 70 (HSP-70), thymosin  $\beta$ 4 (TB4), superoxide dismutase (SOD), and the generation of osteoblasts. These factors are released within the platelet secretome, further highlighting their involvement in osteoblast development [32].

The imbalances in the ubiquitin-proteasome system that are determined by an increased concentration of immunoglobulins is one of the primordial factors that will determine an excess of procoagulant system components. The  $\beta$ 1,  $\beta$ 2 and  $\beta$ 5 subunits are predominantly implied and will be conjugated into  $\beta$ 1i/LMP2,  $\beta$ 2i/MECL-1 and  $\beta$ 5i/LMP7 [33]. Overall, they can form an immunoproteasome that is not reusable and may interact with the elements of the coagulation cascade. The immunocompromising factors like the human immunodeficiency virus 1 (HIV-1) will harshen the state of the patient because it will exhaust the anticoagulant system prior to a surgical intervention (which implies external stimuli) [34]. Compared with humans, the canines have a greater coagulation-anticoagulation system in terms of components. Although it is functionally inferior and has a slower interaction due to a less great interaction surface between the molecules making it less efficient [35].

In order to assess thrombosis in emergency states, the D-dimer biomarker was implemented. It is a reminiscence of the fibrin portion afore the thrombogenesis and less often they can be associated with dysfibrinogenemia [36]. The quantitative aspect of the D-dimers was associated with ter-

minal states like cancer or a chronic cardiovascular disease like a coronaropathy [37].

Fibrinogen was associated with an increased incidence of thrombosis along with additional elements besides the Virchow's triad because of the systemic inflammation and the renin-angiotensin system dysregulations [38]. In the context of COVID-19, fibrin was one of the primordial factors that determined the ground-glass pneumonia in the inferior lobes. Its consolidation was determined by the croupous inflammation that leads to respiratory arrest and death via cardiac failure [39].

The C-reactive protein (CRP) which is a global biomarker for the specific inflammatory states was proven to be associated with the arterial thromboembolism incidence. This in turn is regarded to the atherosclerosis in which the *tunica intima* is swollen and the secretion of inflammatory cytokines or other components takes place into the neointima because of the young myocytes in this layer [40].

A clinically applied biomarker that is widely used is the quantification of platelets count in the bloodstream. An increase in platelet count is often observed, especially in the presence of arterial comorbidities. This elevation is associated with an increased likelihood of platelets coming into contact with arterial walls, leading to microtrauma and potentially contributing to thrombus formation [41]. Conversely, in cases of antithrombin III deficiency, there is a depletion of procoagulant factors, which can increase the risk of arterial thrombosis [42]. The deficiency of protein C or protein S, primarily caused by genetic factors, is another significant factor contributing to the increased incidence of arterial thromboembolism, particularly in young individuals without underlying chronic cardiovascular diseases [43].

Complications associated with arterial thromboembolism are primarily caused by ischemia and its subsequent reactive hypoxia. These complications arise due to restricted blood flow in conditions such as myocardial infarction, ischemic cerebral strokes, and pulmonary embolism. They are common in the COVID-19 patients [44]. The complexity of a biochemical analysis in these patients is thus greater and has numerous inclusion and exclusion criteria.

Matrix metalloproteinases 1 and 8 (MMP-1 and MMP-8), as well as neutrophil gelatinase-associated lipocalin, are commonly used biomarkers for predicting the recurrence and chronicity of comorbidities associated with arterial thromboembolism [45].

Abciximab, a murine monoclonal antibody derived from immunoglobulin G, exerts its pharmacological effects independent of the active centers of platelet membrane receptors. Instead, it forms biochemical bonds with the stable portion of the protein, specifically the  $\beta_3$  perimembranous chains. This mechanism explains its ability to inhibit secretions from endothelial cells, myocardial cells, and leukocytes through interactions with  $\alpha\beta$  or  $\alpha_v\beta_3$  and  $\alpha_m\beta_2$  integrins, respectively.

Eptifibatide, a glycoprotein IIb/IIIa inhibitor, competes with proaggregant molecules such as fibrinogen, von Willebrand factor, and other adhesive ligands for binding to

the GP IIb/IIIa receptor and its associated  $\alpha_v\beta_3$  integrin. By blocking these interactions, eptifibatide prevents platelet aggregation.

Tirofiban, on the other hand, is a selective antagonist for the GP IIb/IIIa receptor and does not exhibit specificity for other integrins. It can be used as a valuable criterion for considering vascular comorbidities when assessing the risk associated with administering the aforementioned pharmacological drugs [46].

Other receptors like P2Y<sub>12</sub> are used for the non-surgical treatment of the arterial thromboembolism; the most notorious being clopidogrel, which can be substituted with prasugrel and ticagrelor, though their superiority in clinical practice was not proven in comparison with clopidogrel. The first-mentioned drug is usually associated with acetylsalicylic acid (aspirin) and heparin [47].

In the past, the treatment was constituted from the administration of vasodilators, thrombolytics, anticoagulants, antibiotics and analgesics. Currently only anticoagulants and thrombolytics are used [48-50]. This pathophysiological particularity determined the topical usage of the anti-inflammatory drugs in prethrombotic cases like osteoarthritis, tendinitis, muscular strains, or muscular retractions [51]. A type II glycopeptide of natural origin, which is called ristocetin from the ristocetin complex that has its active site, labelled „Spontin”, was in past proven to be the cause of platelet aggregation in the von Willebrand disease of platelet type along with the acquired exhaustive thrombocytopenia. Now it is used as a diagnostic hallmark without being applied in the antibiotic-resistant bacterioses [52].

### Venous thromboembolism

The venous thromboembolism has a sudden onset that is none determined by specific causes [2]. The additive factors are obvious, thus being correlated with the venous thromboembolism and are represented by the advanced age, tobacco consumption and increased adiposity [53]. The genetic factors can be primordial in determining the risk for the venous thromboembolism along with an enhancing of the clotting factors activity in the hormonal therapy with oral contraceptives besides other pharmacological drugs. They have genetic polymorphisms and will lead to the prior initiation of the coagulation cascade via the direct, alternative, or lectinic pathway [54]. Drugs that can be deterministic to the hypercoagulable states are systemic estrogen, tamoxifen, corticosteroids, the selective reuptake inhibitors of serotonin, cisplatin, talidomide and lenalidomide [7].

Pregnancy, sepsis (sometimes puerperal), long-time immobilization (trauma, paralysis, sedentary lifestyle) are the figuring main factors in venous thromboembolism development [54-56] along with inflammatory bowel disease and advanced age. The Zahn lines, which are characteristic for the venous thrombus, are due to the stratification of the consequent layers which are composed of erythrocytes and leukocytes, the red color comes from the hemoglobin and the white color due to empty platelets after degranulation in the primary hemostasis [57]. Due to the diverse approaches

employed across multiple disciplines in assessing patients at a population level, there is a potential for an overestimation of venous thromboembolism occurrences [58]. Cancer, congestive cardiac failure, recent surgical interventions, and primary and secondary immunodeficiency are established risk factors for venous thromboembolism. The secondary thrombus formation involves not only platelets but also neutrophil granulocytes, monocytes/macrophages, and exfoliated endothelial cells from the *tunica intima* [53, 57]. Different types of cancer exhibit varying incidences of venous thromboembolism. Breast and urinary bladder cancers have a 3% incidence, while colon and prostate cancers have a higher range of 4-7% incidence. Stomach, lung, ovary, and brain cancers demonstrate a higher incidence range of 10-12%, and pancreatic cancer has the highest incidence at 15% [2]. The recurrence rate of venous thromboembolism over a 10-year period is approximately 30% [55].

The risk factors for venous thromboembolism can be classified into three categories: transient risk factors (synergistic), persistent risk factors (additive), and hereditary risk factors (genetic) [7].

The venous thromboembolism is clinically divided in the peripheral (profound venous) and central (pulmonary thromboembolism) that is frequently incriminated in all thromboembolism, being the leading cause of death [53]. Profound venous thrombosis (PVT) can be acute (<14 days) or chronic (>28 days). The subacute form is characterized by a duration of 14-28 days [57].

Topographically, it has been identified 40% of profound venous thromboembolism cases proximally and 25% distally [2]. Imagistic methods are used in 40% in the pulmonary thromboembolism or in 85% in the profound venous thrombosis [58]. The most frequent form is the profound thromboembolism thus of primary origin and the pulmonary embolism along with the superficial venous thromboembolism are usually complications and sometimes are regarded as primary pathological entities [55].

The most important symptoms in the profound venous thromboembolism are pain, edema, and distal proximal ulceration. In the last years, there is a tendency for increased estimable costs or for the hospitalization of patients with venous thromboembolism. Readmission in the state or private clinics costs more than the primary admission (with 48% more) [59]. The superficial venous thromboembolism is often confounded as a unique clinical entity but is usually regarded as a symptom of the profound venous thromboembolism. The symptoms of the pulmonary venous thromboembolism are apnea, pleuritic chest pain, hemoptysis, tachycardia, or hypoxemia but sometimes severe symptoms like sudden death, shock, hypotension, syncope and confusion may be observed [2]. Existing clinical descriptions do not adequately correspond to the severity states of venous thromboembolism, necessitating the imperative use of clinical intuition to ensure accurate diagnosis and suspicion. In order to facilitate the diagnostic process, there are clinical scores like the Wells score for pulmonary thromboembolism, the Wells score for profound venous thromboembo-

lism and the Geneva score for the pulmonary thromboembolism [58].

There is a tight racial correlation that proves an increased susceptibility for the Afro-American race and obese individuals, though the Afro-Americans have a low obesity incidence. These population groups have increased risks due to genetic determinants and an increased viscosity of the blood related to a decreased permeability of the skin, which can be in turn thinner and less adapted for an interaction with the external factors. Obese individuals are proven to have an increased risk for atherosclerosis that is the main factor that determines thromboembolism. The dominant treatment during hospitalization is the administration of unfractionated heparin and low-molecular weight heparin along with the compression of the lower limbs using compression socks. Recently enoxaparin (a heparin with low-molecular weight) and betrixaban (a direct inhibitor of the Xa factor) have proven to be superior in the clinical trials compared to other drugs. The most important biomarker used in the clinical assessment of the venous thromboembolism are the D-dimers [60]. Other markers like C-reactive protein (CRP), P-selectin and the synthesis of thrombin may be applied [58].

The American Association of Hematology recommends an ambulatory approach in the venous thromboembolism prophylaxis with an international normalized ratio (INR) of 2.0-3.0. The recurrent states need to be treated with vitamin K antagonists, but the stable forms are treatable with direct oral anticoagulants [61]. The treatment length must be within the limits of 3-6 months [2]. Vitamin K along with heparins are preferable in patients with renal insufficiency, antiphospholipid syndrome, and cancer [62].

The monoclonal antibodies can serve as lytic adjuvants because of their amelioration in the intercellular signaling pathways between the circulating leucocytes. The neutralization of the C-reactive protein (CRP), interleukin 6 (IL-6), interleukin (IL-8), interferon- $\gamma$  (IFN- $\gamma$ ), tumor necrosis factor  $\alpha$  - Tumor necrosis factor  $\alpha$  receptor rp55 (TNF- $\alpha$ /TNF- $\alpha$  receptor rp55) and P-selectin by the monoclonal antibodies or the corresponding polyclonal antibodies will induce an ameliorated cellular answer compared to the low entropy of signaling during the venous thrombus lysis. The potentiation of the p53 pathway (during the quinacrine usage) is the pharmacologic alternative during the factor insufficiency [57].

Reperfusion techniques in the venous or pulmonary thromboembolism may be vital in order to prevent the exacerbation of the state thus to the development of a morbid complication. The filters for the vena cava are proven efficient in lowering of a profound venous thromboembolism but may be used only in reserved cases [62, 63]. Mechanical therapy with the compression of the legs is a significant adjuvant factor because it will contribute essentially to the physiological venous blood flow [62]. Bleeding is the most frequent adverse reaction [64].

Pregnant patients are tested using perfusion-ventilation tests in order to avoid the excessive irradiation. The lack of

adequate treatment in profound venous thromboembolism in turn will lead to the confrontation of more severe conditions like pulmonary thromboembolism with congestive heart failure. The symptoms will be hemoptysis, dyspnea, pleuritic chest pain, and hypoxic hypotension. Magnetic resonance imaging has a restriction due to use of gadolinium contrast because it is very time-consuming and can lead to cardiac arrhythmias due to the electromagnetic fields effect. Computed tomography is preferable. Additional biomarkers along with the D-dimers are the soluble form of P-selectin, the first and second clotting factors along with the factor VIII [65]. We observe an increased quantity of interleukin-6 (IL-6) and inter-cellular adhesion molecule 1 (ICAM-1) [57].

In the pediatric population the most important risk factor for the venous thromboembolism is the central venous catheter, but is less often found compared to the adult population, having now a steady increase in incidence [64, 66].

The deficiency of protein C is common, Protein S deficiency is a relatively rare condition, but it is more frequently observed in the Asian population, the mutations for the genes which codify the von Leiden factor (V) are characteristic for the Caucasian population. Hereditary thrombophilia was reported in approximately 8.8% of the studied cohorts in Asia and the deficiency of the antithrombin III was not estimated along with the von Willebrand disease or acquired/hereditary hyperhomocysteinemia. The treatment is the same like in the adult population with a variation in the doses of the pharmacological drugs according to the body-weight [64].

The most notorious complication of the venous thromboembolism is the post-thrombotic syndrome which is chronicled by the venous thromboembolism symptoms (pain, edema and ulceration) with a toleration for the pain feelings and the difficulty to walk while there is a progression for the elephantiasis (like the cardiac insufficiency disabilities). The incidence of the post-thrombotic syndrome varies between 25-50% and is lower in the endovascular interventions favoring pharmacological thrombolysis [57]. This fact will determine the necessity of an individual approach in the diagnostic and treatment options for the venous thromboembolism [67].

### **Molecular biomarkers**

The literature proves that there are no serum biomarkers that may confirm a diagnostic of thromboembolism in a specific manner, no matter of its genesis. Some markers have a high sensitivity with a low specificity thus making them unable to confirm thromboembolism. This context creates the conditions in which additional studies are necessary in order to prove other biochemical markers with diagnostic and prognostic potential. Following, will be described a short list of markers, some of which are experimental.

#### *Protein C*

It is one of the clotting factors that has a similar structure and function with the prothrombin, factors VII, IX and X. It has a light chain and a heavy chain that are interconnected via disulfide bonds formed between the variable cysteine residues [68]. It has a molecular mass of 52.071 Da

and is codified by the PROC gene that is located on 2q14.3 [69]. Its structure is stabilized by the Ca<sup>2+</sup> ions that have an increased affinity for the GLA domain [68]. This protein has nothing to do with the C-reactive protein and is not involved in the inflammatory reactions [70]. During the menstrual cycle, ovulatory phase notorious resistance to the activated protein C was noticed [71]. The lack of protein C or the resistance to the activated protein C is manifested as venous thromboembolism [72].

#### *Protein S*

It is the cofactor of the activated protein C and the tissue factor pathway inhibitor. From a structural overview, it is a glycoprotein that is rich in  $\gamma$ -carboxyglutamate [73]. It has a molecular mass of 75.123 Da and is codified by the PROS1 gene that is localized on the 3q11.1 chromosome band [74]. The anticoagulant effect is due to the fact that it may form glutamyl-heparin bounds in the exits of the factor IXa no matter of its concentration and the activity of the VIII factor [73]. Estrogen is influenced by the concentration of the serum protein S due to this fact we can explain its variation in the patients that administer oral contraceptives and its monthly fluctuation during the menstrual cycle [75]. Like bilirubin, the measurement of protein S is based on the free portion, the portion that is bound with the  $\gamma$ -globulins and is made along with the measurement of protein C. The normal values of these proteins are found in Table 1 [76]. Venous thromboembolism has a significant statistical correlation with protein S concentrations [72].

**Table 1.** Normal concentrations for protein C and protein S.

<b>Protein C</b>	<b>Values (UI/dl)</b>
1-5 years	40-92
6-10 years	45-93
11-16 years	55-110
Adult	64-128
<b>Protein S (total fraction)</b>	<b>Values (UI/dl)</b>
1-5 years	54-118
6-10 years	41-114
11-16 years	52-92
Adult	60-113
<b>Protein S (free fraction)</b>	<b>Values (UI/dl)</b>
1-5 years	21-69
6-10 years	22-62
11-16 years	26-55
Adult	27-61

#### *D-dimers*

D-dimers are the product of fibrin degradation due to its interaction with thrombin. They can become a valuable biomarker in the diagnostic process of arterial and venous thromboembolism [77]. There is no gene which may codify this compound but there are antibodies against D-dimers that are expressed by the corresponding genes, the most important of them being tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ),

CD40 ligand and interleukin 10 (IL-10) [78]. There are no well-established intervals for the D-dimers concentration in blood thus their concentration is often subjective above the values of 0.5 µg/ml [76, 79]. A disadvantage is that D-dimer has a high sensibility but a low specificity thus a low prediction capacity with many false-positive cases [80, 82, 83]. Factors like age, pregnancy, inflammatory diseases, cardiovascular diseases and disseminated intravascular coagulation can make the result even more inconclusive. The measurement of D-dimer necessitates their association with other biomarkers in order to confirm a diagnostic [81-83].

#### *Fibrinogen*

It is a soluble protein that can form bonds with keratin, myosin, and epidermin. It's composed of three chains – A $\alpha$ , B $\beta$  and  $\gamma$  [84] that are codified by the corresponding genes – FGA and FGB localized in a cluster on 4q31.3 and FGG on 4q32.1 [85-87] with a 94.973 Da [85], 55.928 [86] and 51.512 Da molecular masses [87]. The final biochemical structure has  $\alpha$ -helicoidally conformation and 3 domains – A (N-terminal), B and P (C-terminal). Fibrinogen has the capacity to bind Ca<sup>2+</sup> ions. Interacting with thrombin it is cleaved into fibrin [84]. Fibrinogen has markedly increased serum concentration along with an erythrocyte number in venous thromboembolism [88, 89].

#### *Selectin P, Selectin L and Selectin E*

Selectins are peptides that are part of the type C lectin superfamily and are composed of a N-terminal domain that can bind Ca<sup>2+</sup> ions along with an epidermal growth factor (EGF), peptidic tandems and a transmembranary domain [90]. P selectin has a molecular mass of 90.834 Da [91]. L selectin has a molecular mass of 42.187 Da [92] and E-selectin has a molecular mass of 66.655 Da [93]. These proteins are codified by a gene cluster located on the 1q24.2 chromosomal band [91-93]. Selectins are expressed on the surface of the platelets, endotheliocytes and leucocytes. The elevated blood concentrations of the soluble P-selectin forms along with the soluble E-selectin forms can be observed in association of an emerging venous thromboembolism in human study polls. We must mention that E-selectin can be genetically determined for unusual alleles that can predict an increased risk for thromboembolism and are dependent on the menstrual cycle (the luteal phase has its soluble form elevated in plasma) [94-95]. The soluble form of L-selectin was associated with thrombosis events [96].

#### *Interleukins*

They are represented by a series of proteins that are located in the regulatory leukocytes and are responsible for intercellular communication. They are codified and expressed in clusters and furthermore cleaved and stored. We identify 38 types of interleukins (IL 1-38) [97]. It was proven that interleukin 6, interleukin 8 and the monocytic chemoattractant protein 1 (MCP-1) are capable of inducing coagulation events. One of the major interleukins which secretion is low in the thromboembolism events realizes the differential diagnosis with the majority of unspecific inflammatory states is interleukin 10 (IL-10) [98] while leukocytosis is not mandatorily associated [99].

#### *C-reactive protein (CRP)*

It is represented by a pentameric protein synthesized in the liver and is activated by the interaction of interleukin 6 (IL-6) on the genomic structures in inflammatory states [100]. It has a molecular mass of 25.039 Da and is expressed by the CRP gene located on the 1q23.2 chromosome band [101]. For the adult population, concentrations that are lower than 0.3 mg/dl are normal, those that are located between 0.3-1.0 mg/dl are mildly elevated, 1.0-10.0 mg/dl moderately elevated, 10.0-50.0 mg/dl markedly elevated and >50.0 mg/dl is severe elevation [100]. We have proven that it has increased concentrations in venous thromboembolism [102-103] and arterial thrombosis [103].

#### *Thrombin/Antithrombin (TAT)*

Thrombin is represented by an enzyme that is responsible for the fibrin conversion from fibrinogen [104]. Antithrombin III is a glycoprotein composed from 432 aminoacids that inhibit thrombin and thus is part of the serin-dependent protease inhibitor from the serpine group. The  $\alpha$ -antithrombin has a conformation that can bind all the 4 domains of the thrombin while  $\beta$ -antithrombin is able to glycosylate only 3 of them [105]. The balance between the thrombin-antithrombin complex is maintained on the principles exposed in the Michaelis-Menten equation and thus can express the intensity of the platelet aggregation processes and in turn the thrombin generation must be measured without getting an insight into their concentration [104].

#### *Plasma proteins (Albumins, Globulins)*

Albumins are the main protein fraction in the blood plasma, being synthesized in the liver [106]. Globulins are represented by the reminiscent portion that is made out of the  $\alpha_1$ ,  $\alpha_2$ ,  $\beta$  and  $\gamma$  portions that are represented by the immunoglobulins, complement and transport proteins like haptoglobulin, transferrin, ceruloplasmin and many more [107]. The albumin concentrations are variable between 3.5-5 g/dl [106] while globulins have variable values because they are dependent on the total protein fraction and albumin fraction:  $C_{\text{globulins}} = C_{\text{proteins}} - C_{\text{albumins}}$  taking into fact that the total protein fraction has concentrations in the limits of 6-8 g/dl [108]. The low albumin concentrations along with the globulins represent an important indicator for the venous thromboembolism risk in the presence of emerging conditions like the nephrotic syndrome and some physiological states like the low-gravitational field in the cosmic space where the physiology is different [109, 110].

#### *Prostaglandins, Leukotrienes and Nitric Oxide*

Prostaglandins and leukotrienes are eicosanoids, derivatives of the arachidonic acid and polyunsaturated fatty acids. Their synthesis is assured by the cyclooxygenase 1 (COX-1) and cyclooxygenase 2 (COX-2) [111]. It is well known that the nitric oxide has a synergistic effect with the prostaglandins, with an unspecific action while the eicosanoids have their own receptors or can interact with analogue receptors [112]. The main effects of the prostaglandins in the thromboembolism context are the vasodilation (PTGE2), allergic response (PTGD2), musculature contraction and pulmo-

nary vessel contraction (PTGF2) and platelet aggregation inhibition (PTGI2). Leukotrienes like LTB<sub>4</sub> are implied in the turnover if the endotheliocytes while LTC<sub>4</sub>, LTD<sub>4</sub> and LTDE<sub>4</sub> will determine bronchoconstriction, neutrophil extravasation, and vascular response. Nuclear polymorphisms will influence the variability of the functionality of these mediators and the pharmacological response to COX-1 and COX-2 inhibitors (non-steroidal antiinflammatory drugs) [113]. Thromboxanes are not proven to be implied in the thromboembolism in humans, this in turn being proven only for mice species [114, 115].

#### *Matrix metalloproteinases (MMP)*

This family of proteases is represented by Zn<sup>2+</sup> dependent endopeptidases that will control the degradation of the matrix metalloproteinases. Structurally they have a hemopexic domain that is effective for proteolysis in the presence of Zn<sup>2+</sup> ions [116]. They are part of disintegrin and metalloproteinase motif or disintegrin and metalloproteinase thrombospondin motifs and are inhibited by the tissue inhibitor of metalloproteinase 1-4 (TIMP 1-4) which can interact on the C domain of hemopexin [117]. It was proven that myeloperoxidase (MPO) is capable of inhibiting or activating matrix metalloproteinases that are dependent on their subtype and the structure of the C domain of hemopexin [118]. Matrix metalloproteinase 2 (MMP-2) will be activated by the myeloperoxidase (MPO) and will exacerbate the progression of the venous thromboembolism [118, 119] and matrix metalloproteinases 1 (MMP-1) will be regulated only by the tissue inhibitor of metalloproteinase 1-4 (TIMP 1-4) [117, 119]. In mice, the proaggregant action of MMP-9 and MMP-14 was proven without being regarded for the human species [120].

Our comprehension of thromboembolism has advanced, allowing for a more convenient interpretation of its relationship with systemic inflammation, metabolic changes, and other pathophysiological conditions. While these markers offer promising results, relying on any one of them alone may lead to false-positive or false-negative outcomes.

To ensure an accurate diagnostic or prognostic process, it is essential to apply these markers in combination. Further studies are necessary to meet the requirements of medical laboratories and enhance their utility.

#### **Conclusions**

(1) Although arterial and venous thromboembolism typically share common biomarkers, certain molecules may exhibit distinctive characteristics, enabling differential diagnosis. However, their practical applicability in clinical settings is limited.

(2) The majority of biochemical compounds utilized as biomarkers demonstrate strong interrelationships, allowing for the identification of cascade patterns of expression in both physiological and pathophysiological conditions.

(3) Understanding the structural aspects and mechanisms of action within the procoagulant cascade, in conjunction with biomarkers, is crucial for determining an appropriate treatment strategy.

#### **Competing interests**

None declared.

#### **Authors' contribution**

DC conceptualized and realized the study. EP revised, analyzed, and redacted critically the content of the study. All authors revised and approved the final version of the manuscript.

#### **References**

- Lippi G, Cervellini G, Franchini M, Favalaro EJ. Biochemical markers for the diagnosis of venous thromboembolism: the past, present and future. *J Thromb Thrombolysis*. 2010 Nov;30(4):459-71. doi: 10.1007/s11239-010-0460-x.
- Khan F, Tritschler T, Kahn SR, Rodger MA. Venous thromboembolism. *Lancet*. 2021 Jul 3;398(10294):64-77. doi: 10.1016/S0140-6736(20)32658-1.
- Berghaus TM, Haeckel T, Behr W, Wehler M, von Scheidt W, Schwaiblmair M. Central thromboembolism is a possible predictor of right heart dysfunction in normotensive patients with acute pulmonary embolism. *Thromb Res*. 2010 Sep;126(3):e201-5. doi: 10.1016/j.thromres.2010.06.009.
- Visweshwar N, Jaglal M, Sokol L, Djulbegovic B. Hematological malignancies and arterial thromboembolism. *Indian J Hematol Blood Transfus*. 2019 Oct;35(4):611-624. doi: 10.1007/s12288-019-01085-x.
- Ashorobi D, Ameer MA, Fernandez R. Thrombosis [updated 2022 Sep 5]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan- [cited 2023 Mar 30]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK538430/>
- Luis Fuentes V. Arterial thromboembolism: risks, realities and a rational first-line approach. *J Feline Med Surg*. 2012 Jul;14(7):459-70. doi: 10.1177/1098612X12451547.
- Phillippe HM. Overview of venous thromboembolism. *Am J Manag Care*. 2017 Dec;23(20 Suppl):S376-S382.
- Andersen LV, Lip GY, Lindholt JS, Frost L. Upper limb arterial thromboembolism: a systematic review on incidence, risk factors, and prognosis, including a meta-analysis of risk-modifying drugs. *J Thromb Haemost*. 2013 May;11(5):836-44. doi: 10.1111/jth.12181.
- Etkin Y, Conway AM, Silpe J, Qato K, Carroccio A, Manvar-Singh P, Giangola G, Deitch JS, Davila-Santini L, Schor JA, Singh K, Mussa FF, Landis GS. Acute arterial thromboembolism in patients with COVID-19 in the New York City area. *Ann Vasc Surg*. 2021 Jan;70:290-294. doi: 10.1016/j.avsg.2020.08.085.
- Porfidia A, Valeriani E, Pola R, Porreca E, Rutjes AWS, Di Nisio M. Venous thromboembolism in patients with COVID-19: systematic review and meta-analysis. *Thromb Res*. 2020 Dec;196:67-74. doi: 10.1016/j.thromres.2020.08.020.
- Wang J, Kim YD, Kim CH. Incidence and risk of various types of arterial thromboembolism in patients with cancer. *Mayo Clin Proc*. 2021 Mar;96(3):592-600. doi: 10.1016/j.mayocp.2020.05.045.
- Kudo D, Goto T, Uchimido R, et al. Coagulation phenotypes in sepsis and effects of recombinant human thrombomodulin: an analysis of three multicentre observational studies. *Crit Care*. 2021;25(1):114. <https://doi.org/10.1186/s13054-021-03541-5>.
- Ștefăneț M. Anatomia omului [Human anatomy]. Vol. 2. Chișinău: Medicina; 2018. 414 p. Romanian.
- Scott BA, Garg K, Johnson W, Al-Ajam M, Patalano P, Rotella V, Edwards JA, Aboushi H, Lee P, Daniel M, Rancy S,



- Heimann D. Upper extremity arterial thromboembolism in a coronavirus patient: a case report. *SN Compr Clin Med.* 2021;3(1):273-278. doi: 10.1007/s42399-020-00694-2.
15. Mayer D. *Essential evidence-based medicine*. 2nd ed. New York: Cambridge University Press; 2010. 442 p.
  16. Senst B, Tadi P, Basit H, et al. Hypercoagulability [updated 2022 Sep 26]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan- [cited 2023 Mar 30]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK538251/>
  17. Kushner A, West WP, Khan Suheb MZ, et al. Virchow triad [updated 2022 Nov 2]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan- [cited 2023 Mar 30]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK539697/>
  18. Schafer AI. The hypercoagulable states. *Ann Intern Med.* 1985 Jun;102(6):814-28. doi: 10.7326/0003-4819-102-6-814.
  19. Subramaniam S, Scharrer I. Procoagulant activity during viral infections. *Front Biosci (Landmark Ed).* 2018 Jan 1;23(6):1060-1081. doi: 10.2741/4633.
  20. Marchel H, Wroblewska M. Bloodstream infections – etiology and current microbiological diagnostics. *Pol Ann Med.* 2022;29(1):83-87. doi: 10.29089/2021.21.00193.
  21. Moldotashev IK, Taalaibekova TT, Kudaibergenova NT, Orozaliev MD, Usubaliev NN, Kydyralieva RB. Influence of heliogeophysical factors on patients with myocardial infarction. *Pol Ann Med.* 2022;29(1):38-43. doi: 10.29089/2021.21.00190.
  22. Storozhuk O, Storozhuk L, Dovgalyuk T, Storozhuk N, Androsova N, Storozhuk B. New method in assessment of blood plasma coagulation balance. *Pol Ann Med.* 2022;29(2):94-98. doi: 10.29089/2021.21.00197.
  23. Leroyer AS, Tedgui A, Boulanger CM. Microparticles and type 2 diabetes. *Diabetes Metab.* 2008 Feb;34 Suppl 1:S27-32. doi: 10.1016/S1262-3636(08)70100-9.
  24. Leroyer AS, Tedgui A, Boulanger CM. Role of microparticles in atherothrombosis. *J Intern Med.* 2008 May;263(5):528-37. doi: 10.1111/j.1365-2796.2008.01957.x.
  25. Morel O, Toti F, Hugel B, Bakouboula B, Camoin-Jau L, Dignat-George F, Freyssinet JM. Procoagulant microparticles: disrupting the vascular homeostasis equation? *Arterioscler Thromb Vasc Biol.* 2006 Dec;26(12):2594-604. doi: 10.1161/01.ATV.0000246775.14471.26.
  26. Morel O, Morel N, Freyssinet JM, Toti F. Platelet microparticles and vascular cells interactions: a checkpoint between the haemostatic and thrombotic responses. *Platelets.* 2008 Feb;19(1):9-23. doi: 10.1080/09537100701817232.
  27. Saboor M, Ayub Q, Ilyas S, Moinuddin. Platelet receptors: an instrumental of platelet physiology. *Pak J Med Sci.* 2013 May;29(3):891-6. doi: 10.12669/pjms.293.3497.
  28. Liși L, Pavlovschi E. *Biochimie medicala [Medical biochemistry]*. 3rd ed. Chisinau; 2019. p. 686-690. Romanian.
  29. Petersen KR. Pharmacodynamic effects of oral contraceptive steroids on biochemical markers for arterial thrombosis. Studies in non-diabetic women and in women with insulin-dependent diabetes mellitus. *Dan Med Bull.* 2002 Feb;49(1):43-60.
  30. Baidildinova G, Nagy M, Jurk K, Wild PS, ten Cate H, van der Meijden PEJ. Soluble platelet release factors as biomarkers for cardiovascular disease. *Front Cardiovasc Med.* 2021;8:684920. doi:10.3389/fcvm.2021.684920.
  31. Del Amo C, Perez-Valle A, Atilano L, Andia I. Unraveling the signaling secretome of platelet-rich plasma: towards a better understanding of its therapeutic potential in knee osteoarthritis. *J Clin Med.* 2022 Jan 18;11(3):473. doi: 10.3390/jcm11030473.
  32. Tiedemann K, Tsao S, Komarova SV. Platelets and osteoblasts: secretome connections. *Am J Physiol Cell Physiol.* 2022 Aug 1;323(2):C347-C353. doi: 10.1152/ajpcell.00187.2022.
  33. Colberg L, Cammann C, Greinacher A, Seifert U. Structure and function of the ubiquitin-proteasome system in platelets. *J Thromb Haemost.* 2020 Apr;18(4):771-780. doi: 10.1111/jth.14730.
  34. Yang Y, Liu W, Hu D, Su R, Ji M, Huang Y, Shereen MA, Xu X, Luo Z, Zhang Q, Liu F, Wu K, Liu Y, Wu J. HIV-1 Nef interacts with LMP7 to attenuate immunoproteasome formation and major histocompatibility complex class I antigen presentation. *mBio.* 2020 Oct 27;11(5):e02221-19. doi: 10.1128/mBio.02221-19.
  35. Cremer SE, Catalfamo JL, Goggs R, Seemann SE, Kristensen AT, Szklanna PB, Maguire PB, Brooks MB. The canine activated platelet secretome (CAPS): a translational model of thrombin-evoked platelet activation response. *Res Pract Thromb Haemost.* 2020 Dec 3;5(1):55-68. doi: 10.1002/rth2.12450.
  36. Kleinegris MC, ten Cate H, ten Cate-Hoek AJ. D-dimer as a marker for cardiovascular and arterial thrombotic events in patients with peripheral arterial disease. A systematic review. *Thromb Haemost.* 2013 Aug;110(2):233-43. doi: 10.1160/TH13-01-0032.
  37. Simes J, Robledo KP, White HD, Espinoza D, Stewart RA, Sullivan DR, Zeller T, Hague W, Nestel PJ, Glasziou PP, Keech AC, Elliott J, Blankenberg S, Tonkin AM; LIPID Study Investigators. D-dimer predicts long-term cause-specific mortality, cardiovascular events, and cancer in patients with stable coronary heart disease: LIPID Study. *Circulation.* 2018 Aug 14;138(7):712-723. doi: 10.1161/CIRCULATIONAHA.117.029901.
  38. Kryczka KE, Kruk M, Demkow M, Lubiszewska B. Fibrinogen and a triad of thrombosis, inflammation, and the renin-angiotensin system in premature coronary artery disease in women: a new insight into sex-related differences in the pathogenesis of the disease. *Biomolecules.* 2021 Jul 15;11(7):1036. doi: 10.3390/biom11071036.
  39. Khismatullin RR, Ponomareva AA, Nagaswami C, Ivaeva RA, Montone KT, Weisel JW, Litvinov RI. Pathology of lung-specific thrombosis and inflammation in COVID-19. *J Thromb Haemost.* 2021 Dec;19(12):3062-3072. doi: 10.1111/jth.15532.
  40. Horvei LD, Grimnes G, Hindberg K, Mathiesen EB, Njølstad I, Wilsgaard T, Hansen JB. C-reactive protein, obesity, and the risk of arterial and venous thrombosis. *J Thromb Haemost.* 2016;14(8):1561-1571. doi: 10.1111/jth.13369.
  41. Warny M, Helby J, Birgens HS, Bojesen SE, Nordestgaard BG. Arterial and venous thrombosis by high platelet count and high hematocrit: 108,521 individuals from the Copenhagen General Population Study. *J Thromb Haemost.* 2019;17(11):1898-1911. doi:10.1111/jth.14574.
  42. Nishimura M, Shimada J, Ito K, Kawachi H, Nishiyama K. Acute arterial thrombosis with antithrombin III deficiency in nephrotic syndrome: report of a case. *Surg Today.* 2000;30(7):663-6. doi: 10.1007/s005950070110.
  43. Mahmoodi BK, Brouwer JL, Veeger NJ, van der Meer J. Hereditary deficiency of protein C or protein S confers increased risk of arterial thromboembolic events at a young age: results from a large family cohort study. *Circulation.* 2008;118(16):1659-1667. doi: 10.1161/circulationaha.108.780759.

44. Lalor N, Raffaelli A, Bottini F, Scapellato JL, Banos N, Pezzati L, Intile D, Longo F. Major arterial and venous thromboembolic complications in patients with COVID-19. *Eur Heart J*. 2021;42(Suppl 1):ehab724.1921. <https://doi.org/10.1093/eurheartj/ehab724.1921>.
45. de Franciscis S, Gallelli L, Amato B, Butrico L, Rossi A, Bufone G, Calì FG, De Caridi G, Grande R, Serra R. Plasma MMP and TIMP evaluation in patients with deep venous thrombosis: could they have a predictive role in the development of post-thrombotic syndrome? *Int Wound J*. 2016 Dec;13(6):1237-1245. doi: 10.1111/iwj.12489.
46. Hashemzadeh M, Furukawa M, Goldsberry S, Movahed MR. Chemical structures and mode of action of intravenous glycoprotein IIb/IIIa receptor blockers: a review. *Exp Clin Cardiol*. 2008;13(4):192-7.
47. Patti G, Micieli G, Cimmiello C, Bolognese L. The role of clopidogrel in 2020: a reappraisal. *Cardiovasc Ther*. 2020 Mar 16;2020:8703627. doi: 10.1155/2020/8703627.
48. Phillips DR, Conley PB, Sinha U, Andre P. Therapeutic approaches in arterial thrombosis. *J Thromb Haemost*. 2005 Aug;3(8):1577-89. doi: 10.1111/j.1538-7836.2005.01418.x.
49. Mackman N. Triggers, targets and treatments for thrombosis. *Nature*. 2008 Feb 21;451(7181):914-8. doi: 10.1038/nature06797.
50. Hish GA Jr, Diaz JA, Hawley AE, Myers DD Jr, Lester PA. Effects of analgesic use on inflammation and hematology in a murine model of venous thrombosis. *J Am Assoc Lab Anim Sci*. 2014 Sep;53(5):485-93.
51. Derry S, Wiffen PJ, Kalso EA, Bell RF, Aldington D, Phillips T, Gaskell H, Moore RA. Topical analgesics for acute and chronic pain in adults - an overview of Cochrane Reviews. *Cochrane Database Syst Rev*. 2017 May 12;5(5):CD008609. doi: 10.1002/14651858.CD008609.pub2.
52. Butler MS, Hansford KA, Blaskovich MA, Halai R, Cooper MA. Glycopeptide antibiotics: back to the future. *J Antibiot (Tokyo)*. 2014 Sep;67(9):631-44. doi: 10.1038/ja.2014.111.
53. Gregson J, Kaptoge S, Bolton T, Pennells L, Willeit P, Burgess S, et al. Cardiovascular risk factors associated with venous thromboembolism. *JAMA Cardiol*. 2019 Feb 1;4(2):163-173. doi: 10.1001/jamacardio.2018.4537.
54. Nicholson M, Chan N, Bhagirath V, Ginsberg J. Prevention of venous thromboembolism in 2020 and beyond. *J Clin Med*. 2020 Aug 1;9(8):2467. doi: 10.3390/jcm9082467.
55. Heit JA. Epidemiology of venous thromboembolism. *Nat Rev Cardiol*. 2015 Aug;12(8):464-74. doi: 10.1038/nrcardio.2015.83.
56. Bruni-Fitzgerald KR. Venous thromboembolism: an overview. *J Vasc Nurs*. 2015 Sep;33(3):95-9. doi: 10.1016/j.jvn.2015.02.001.
57. Mukhopadhyay S, Johnson TA, Duru N, Buzza MS, Pawar NR, Sarkar R, Antalis TM. Fibrinolysis and inflammation in venous thrombus resolution. *Front Immunol*. 2019 Jun 14;10:1348. doi: 10.3389/fimmu.2019.01348.
58. Liederman Z, Chan N, Bhagirath V. Current challenges in diagnosis of venous thromboembolism. *J Clin Med*. 2020 Oct 29;9(11):3509. doi: 10.3390/jcm9113509.
59. Fernandez MM, Hogue S, Preblick R, Kwong WJ. Review of the cost of venous thromboembolism. *Clinicoecon Outcomes Res*. 2015 Aug 28;7:451-62. doi: 10.2147/CEOR.S85635.
60. Serhal M, Barnes GD. Venous thromboembolism: a clinician update. *Vasc Med*. 2019 Apr;24(2):122-131. doi: 10.1177/1358863X18821159.
61. Ortel TL, Neumann I, Ageno W, Beyth R, Clark NP, Cuker A, et al. American Society of Hematology 2020 guidelines for management of venous thromboembolism: treatment of deep vein thrombosis and pulmonary embolism. *Blood Adv*. 2020 Oct 13;4(19):4693-4738. doi: 10.1182/bloodadvances.2020001830.
62. Becattini C, Agnelli G. Acute treatment of venous thromboembolism. *Blood*. 2020 Jan 30;135(5):305-316. doi: 10.1182/blood.2019001881.
63. Wells PS, Forgie MA, Rodger MA. Treatment of venous thromboembolism. *JAMA*. 2014 Feb 19;311(7):717-28. doi: 10.1001/jama.2014.65. Erratum in: *JAMA*. 2014 Jun 25;311(24):2545.
64. Hong J, Ahn SY, Lee YJ, Lee JH, Han JW, Kim KH, et al. Updated recommendations for the treatment of venous thromboembolism. *Blood Res*. 2021 Mar 31;56(1):6-16. doi: 10.5045/br.2021.2020083.
65. Patel H, Sun H, Hussain AN, Vakde T. Advances in the diagnosis of venous thromboembolism: a literature review. *Diagnostics (Basel)*. 2020 Jun 2;10(6):365. doi: 10.3390/diagnostics10060365.
66. Citla Sridhar D, Abou-Ismaïl MY, Ahuja SP. Central venous catheter-related thrombosis in children and adults. *Thromb Res*. 2020;187:103-112. doi: 10.1016/j.thromres.2020.01.017.
67. Tritschler T, Kraaijpoel N, Le Gal G, Wells PS. Venous thromboembolism: advances in diagnosis and treatment. *JAMA*. 2018 Oct 16;320(15):1583-1594. doi: 10.1001/jama.2018.14346. Erratum in: *JAMA*. 2018 Dec 18;320(23):2486.
68. Stojanovski BM, Pelc LA, Zuo X, Di Cera E. Zymogen and activated protein C have similar structural architecture. *J Biol Chem*. 2020 Nov 6;295(45):15236-15244. doi: 10.1074/jbc.RA120.014789.
69. GeneCards - the human gene database. PROC Gene [Internet]. Rehovot: Weizmann Institute of Science; 2023- [cited 2023 Mar 30]. Available from: <https://www.genecards.org/cgi-bin/carddisp.pl?gene=PROC>
70. McFadyen JD, Zeller J, Potempa LA, Pietersz GA, Eisenhardt SU, Peter K. C-reactive protein and its structural isoforms: an evolutionary conserved marker and central player in inflammatory diseases and beyond. *Subcell Biochem*. 2020;94:499-520. doi: 10.1007/978-3-030-41769-7\_20.
71. Rühl H, Schröder L, Müller J, Sukhitasvili S, Welz J, Kuhn WC, et al. Impact of hormone-associated resistance to activated protein C on the thrombotic potential of oral contraceptives: a prospective observational study. *PLoS One*. 2014;9(8):e105007. <https://doi.org/10.1371/journal.pone.0105007>
72. Bucciarelli P, Rosendaal FR, Tripodi A, Mannucci PM, De Stefano V, Palareti G, et al. Risk of venous thromboembolism and clinical manifestations in carriers of antithrombin, protein C, protein S deficiency, or activated protein C resistance: a multicenter collaborative family study. *Arterioscler Thromb Vasc Biol*. 1999 Apr;19(4):1026-33. doi: 10.1161/01.atv.19.4.1026.
73. Majumder R, Nguyen T. Protein S: function, regulation, and clinical perspectives. *Curr Opin Hematol*. 2021 Sep 1;28(5):339-344. doi: 10.1097/MOH.0000000000000663.
74. GeneCards - the human gene database. PROS1 Gene [Internet]. Rehovot: Weizmann Institute of Science; 2023- [cited 2023 Mar 30]. Available from: <https://www.genecards.org/cgi-bin/carddisp.pl?gene=PROS1>
75. Archer DF, Thomas MA, Conard J, Merkatz RB, Creasy GW, Roberts K, Plagianos M, Blithe D, Sitruk-Ware R. Impact on

- hepatic estrogen-sensitive proteins by a 1-year contraceptive vaginal ring delivering Nestorone® and ethinyl estradiol. *Contraception*. 2016 Jan;93(1):58-64. doi: 10.1016/j.contraception.2015.09.008.
76. Padda IS, Patel P, Citla Sridhar D. Protein S and C [updated 2022 May 8]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan- [cited 2023 Mar 30]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK557814/>
  77. Johnson ED, Schell JC, Rodgers GM. The D-dimer assay. *Am J Hematol*. 2019 Jul;94(7):833-839. doi: 10.1002/ajh.25482.
  78. GeneCards – the human gene database. D-dimer antibodies related-genes [Internet]. Rehovot: Weizmann Institute of Science; 2023- [cited 2023 Mar 30]. Available from: <https://www.genecards.org/Search/Keyword?queryString=D-Dimer%20antibodies>
  79. Bounds EJ, Kok SJ. D Dimer [updated 2022 Jul 11]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan- [cited 2023 Mar 30]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK431064/>
  80. Anghel L, Sascău R, Radu R, Stătescu C. From classical laboratory parameters to novel biomarkers for the diagnosis of venous thrombosis. *Int J Mol Sci*. 2020 Mar 11;21(6):1920. doi: 10.3390/ijms21061920.
  81. Kabrhel C, Mark Courtney D, Camargo CA Jr, Plewa MC, Nordenholz KE, Moore CL, Richman PB, Smithline HA, Beam DM, Kline JA. Factors associated with positive D-dimer results in patients evaluated for pulmonary embolism. *Acad Emerg Med*. 2010 Jun;17(6):589-97. doi: 10.1111/j.1553-2712.2010.00765.x.
  82. Karny-Epstein N, Abuhasira R, Grossman, A. Current use of D-dimer for the exclusion of venous thrombosis in hospitalized patients. *Sci Rep*. 2022;12(1):12376. <https://doi.org/10.1038/s41598-022-16515-6>.
  83. Kearon C, de Wit K, Parpia S, Schulman S, Spencer FA, Sharma S, et al. Diagnosis of deep vein thrombosis with D-dimer adjusted to clinical probability: prospective diagnostic management study. *BMJ*. 2022;376:e067378. doi: 10.1136/bmj-2021-067378.
  84. Weisel JW, Litvinov RI. Fibrin formation, structure and properties. *Subcell Biochem*. 2017;82:405-456. doi: 10.1007/978-3-319-49674-0\_13.
  85. GeneCards – the human gene database. FGA Gene [Internet]. Rehovot: Weizmann Institute of Science; 2023- [cited 2023 Mar 30]. Available from: <https://www.genecards.org/cgi-bin/carddisp.pl?gene=FGA>
  86. GeneCards – the human gene database. FGB Gene [Internet]. Rehovot: Weizmann Institute of Science; 2023- [cited 2023 Mar 30]. Available from: <https://www.genecards.org/cgi-bin/carddisp.pl?gene=FGB>
  87. GeneCards – the human gene database. FGG Gene [Internet]. Rehovot: Weizmann Institute of Science; 2023- [cited 2023 Mar 30]. Available from: <https://www.genecards.org/cgi-bin/carddisp.pl?gene=FGG>
  88. Tsai AW, Cushman M, Rosamond WD, Heckbert SR, Tracy RP, Aleksic N, Folsom AR. Coagulation factors, inflammation markers, and venous thromboembolism: the longitudinal investigation of thromboembolism etiology (LITE). *Am J Med*. 2002 Dec 1;113(8):636-42. doi: 10.1016/s0002-9343(02)01345-1.
  89. Aleman MM, Walton BL, Byrnes JR, Wolberg AS. Fibrinogen and red blood cells in venous thrombosis. *Thromb Res*. 2014 May;133 Suppl 1(01):S38-40. doi: 10.1016/j.thromres.2014.03.017.
  90. McEver RP. Selectins: initiators of leucocyte adhesion and signalling at the vascular wall. *Cardiovasc Res*. 2015;107(3):331-339. <https://doi.org/10.1093/cvr/cvv154>.
  91. GeneCards – the human gene database. SELP Gene [Internet]. Rehovot: Weizmann Institute of Science; 2023- [cited 2023 Mar 30]. Available from: <https://www.genecards.org/cgi-bin/carddisp.pl?gene=SELP>
  92. GeneCards – the human gene database. SELL Gene [Internet]. Rehovot: Weizmann Institute of Science; 2023- [cited 2023 Mar 30]. Available from: <https://www.genecards.org/cgi-bin/carddisp.pl?gene=SELL>
  93. GeneCards – the human gene database. SELE Gene [Internet]. Rehovot: Weizmann Institute of Science; 2023- [cited 2023 Mar 30]. Available from: <https://www.genecards.org/cgi-bin/carddisp.pl?gene=SELE>
  94. Purdy M, Obi A, Myers D, Wakefield T. P- and E- selectin in venous thrombosis and non-venous pathologies. *J Thromb Haemost*. 2022 May;20(5):1056-1066. doi: 10.1111/jth.15689.
  95. Ay C, Simanek R, Vormittag R, Dunkler D, Alguel G, Koder S, Kornek G, Marosi C, Wagner O, Zielinski C, Pabinger I. High plasma levels of soluble P-selectin are predictive of venous thromboembolism in cancer patients: results from the Vienna Cancer and Thrombosis Study (CATS). *Blood*. 2008 Oct 1;112(7):2703-8. doi: 10.1182/blood-2008-02-142422.
  96. Watany MM, Abdou S, Elkolaly R, Elgharbawy N, Hodeib H. Evaluation of admission levels of P, E and L selectins as predictors for thrombosis in hospitalized COVID-19 patients. *Clin Exp Med*. 2022;22(4):567-575. <https://doi.org/10.1007/s10238-021-00787-9>.
  97. Akdis M, Aab A, Altunbulakli C, Azkur K, Costa RA, Cramer R, et al. Interleukins (from IL-1 to IL-38), interferons, transforming growth factor  $\beta$ , and TNF- $\alpha$ : receptors, functions, and roles in diseases. *J Allergy Clin Immunol*. 2016;138(4):984-1010. doi: 10.1016/j.jaci.2016.06.033.
  98. Poredos P, Jezovnik MK. In patients with idiopathic venous thrombosis, interleukin-10 is decreased and related to endothelial dysfunction. *Heart Vessels*. 2011;26(6):596-602. doi: 10.1007/s00380-010-0111-3.
  99. Sexton T, Smyth SS. Novel mediators and biomarkers of thrombosis. *J Thromb Thrombolysis*. 2014 Jan;37(1):1-3. doi: 10.1007/s11239-013-1034-5.
  100. Nehring SM, Goyal A, Patel BC. C Reactive Protein [updated 2022 Jul 18]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan- [cited 2023 Mar 30]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK441843/>
  101. GeneCards – the human gene database. CRP [Internet]. Rehovot: Weizmann Institute of Science; 2023- [cited 2023 Mar 30]. Available from: <https://www.genecards.org/cgi-bin/carddisp.pl?gene=CRP>
  102. Grimnes G, Isaksen T, Tichelaar YIGV, Brox J, Brækkan SK, Hansen JB. C-reactive protein and risk of venous thromboembolism: results from a population-based case-cross-over study. *Haematologica*. 2018 Jul;103(7):1245-1250. doi: 10.3324/haematol.2017.186957.
  103. Horvei LD, Grimnes G, Hindberg K, Mathiesen EB, Njølstad I, Wilsgaard T, Brox J, Brækkan SK, Hansen JB. C-reactive protein, obesity, and the risk of arterial and venous thrombosis. *J Thromb Haemost*. 2016;14(8):1561-71. doi: 10.1111/jth.13369.
  104. Larsen JB, Hvas AM. Thrombin: a pivotal player in hemostasis and beyond. *Semin Thromb Hemost*. 2021 Oct;47(7):759-774. doi: 10.1055/s-0041-1727116.
  105. Hsu E, Moosavi L. Biochemistry, Antithrombin III [update

- ed 2022 Sep 12]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan- [cited 2023 Mar 30]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK545295/>
- 106.Moman RN, Gupta N, Varacallo M. Physiology, Albumin [updated 2022 Dec 26]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-[cited 2023 Mar 30]. Available from: <https://www.statpearls.com/articlelibrary/viewarticle/17328/>
  - 107.Hoo T, Lim EM, John M, D'Orsogna L, McLean-Tookey A. Calculated globulin as a screening tool for hypogammaglobulinaemia or paraproteins in hospitalized patients. *Ann Clin Biochem.* 2021;58(3):236-243. doi: 10.1177/0004563221989737.
  - 108.Busher JT. Serum albumin and globulin. In: Walker HK, Hall WD, Hurst JW, editors. *Clinical methods: the history, physical, and laboratory examinations.* 3rd ed. Boston: Butterworths; 1990. Chapter 101.
  - 109.Gyاملani G, Molnar MZ, Lu JL, Sumida K, Kalantar-Zadeh K, Kovesdy CP. Association of serum albumin level and venous thromboembolic events in a large cohort of patients with nephrotic syndrome. *Nephrol Dial Transplant.* 2017 Jan 1;32(1):157-164. doi: 10.1093/ndt/gfw227.
  - 110.Zwart SR, Auñón-Chancellor SM, Heer M, Melin MM, Smith SM. Albumin, oral contraceptives, and venous thromboembolism risk in astronauts. *J Appl Physiol.* 2022 May 1;132(5):1232-1239. doi: 10.1152/jappphysiol.00024.2022.
  - 111.Jara-Gutiérrez Á, Baladrón V. The role of prostaglandins in different types of cancer. *Cells.* 2021 Jun 13;10(6):1487. doi: 10.3390/cells10061487.
  - 112.Broeders MA, Tangelder GJ, Slaaf DW, Reneman RS, Egbrink MG. Endogenous nitric oxide and prostaglandins synergistically counteract thromboembolism in arterioles but not in venules. *Arterioscler Thromb Vasc Biol.* 2001 Jan;21(1):163-9. doi: 10.1161/01.atv.21.1.163.
  - 113.Cornejo-García JA, Perkins JR, Jurado-Escobar R, García-Martín E, Agúndez JA, Viguera E, et al. Pharmacogenomics of prostaglandin and leukotriene receptors. *Front Pharmacol.* 2016;7:316. doi: 10.3389/fphar.2016.00316.
  - 114.Tarantino E, Amadio P, Squellerio I, Porro B, Sandrini L, Turnu L, Cavalca V, Tremoli E, Barbieri SS. Role of thromboxane-dependent platelet activation in venous thrombosis: Aspirin effects in mouse model. *Pharmacol Res.* 2016 May;107:415-425. doi: 10.1016/j.phrs.2016.04.001.
  - 115.Kashiwagi H, Yuhki KI, Imamichi Y, Kojima F, Kumei S, Tasaki Y, Narumiya S, Ushikubi F. Prostaglandin F<sub>2</sub>α facilitates platelet activation by acting on prostaglandin E2 receptor subtype EP3 and thromboxane A2 receptor TP in mice. *Thromb Haemost.* 2019 Aug;119(8):1311-1320. doi: 10.1055/s-0039-1688906.
  - 116.Cui N, Hu M, Khalil RA. Biochemical and biological attributes of matrix metalloproteinases. *Prog Mol Biol Transl Sci.* 2017;147:1-73. doi: 10.1016/bs.pmbts.2017.02.005.
  - 117.Cabral-Pacheco GA, Garza-Veloz I, Castruita-De la Rosa C, Ramirez-Acuña JM, Perez-Romero BA, Guerrero-Rodríguez JF, Martínez-Avila N, Martínez-Fierro ML. The Roles of Matrix Metalloproteinases and Their Inhibitors in Human Diseases. *International Journal of Molecular Sciences.* 2020; 21(24):9739. <https://doi.org/10.3390/ijms21249739>
  - 118.Zhang Y, Dong H, Seeburg DP, Wojtkiewicz GR, Waterman P, Pulli B, Forghani R, Ali M, Iwamoto Y, Swirski FK, Chen JW. Multimodal molecular imaging demonstrates myeloperoxidase regulation of matrix metalloproteinase activity in neuroinflammation. *Mol Neurobiol.* 2019 Feb;56(2):954-962. doi: 10.1007/s12035-018-1137-2.
  - 119.Zhang T, Li Q, Wang L, Li G. Expression variations and clinical significance of MMP-1, MMP-2 and inflammatory factors in serum of patients with deep venous thrombosis of lower extremity. *Exp Ther Med.* 2019 Jan;17(1):181-186. doi: 10.3892/etm.2018.6922.
  - 120.Mastenbroek TG, Feijge MA, Kremers RM, van den Bosch MT, Swieringa F, De Groef L, Moons L, Bennett C, Ghevaert C, Johnson JL, van der Meijden PE, Cossemans JM. Platelet-associated matrix metalloproteinases regulate thrombus formation and exert local collagenolytic activity. *Arterioscler Thromb Vasc Biol.* 2015 Dec;35(12):2554-61. doi: 10.1161/ATVBAHA.115.306153.