ELEMENTS OF VIRCHOW’S TRIAD IN THE PREVENTION AND TREATMENT OF VENOUS THROMBOSIS

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In the mid 19-th century Rudolf Virchow (1) determined three major causes of thrombosis: blood flow disturbances, vascular wall changes and changes in the composition of blood, which run by the name „Virchow’s triad”. During the past 150 years investigations considering venous thrombosis were mostly directed to the latter issue: coagulation disturbances. Based on experimental and clinical trials published during the past decades one can come to the conclusion that prophylaxis and treatment of venous thrombosis should also include the remaining two elements of the triad: lower extremity venous blood flow and vascular wall inflammatory reactions.

In accordance with current opinions concerning the physiology of the coagulation system, its activation is possible by means of the endogenous pathway following the activation of Hageman’s factor, or by means of the exogenous pathway following the binding of factor VII and proconvertin with the tissue factor. Factor VII is synthesized inside the hepatocytes and circulates in its inactive form. Factor VII combines with the tissue factor forming a complex, assuming the form of a protease, activating the enzymatic pathway of consecutive proteases until the creation of thrombin, which converts fibrinogen into fibrin. The following question arises: How is contact possible between the tissue factor and the circulating factor VII without mechanical vascular damage?

Opinions concerning the origin of the tissue factor present in the circulating blood are non-uniform (2, 3). According to Myers and Wakefield (4), leucocytes are the source of the above-mentioned factor. Prescott and co-authors (5) observed the presence of the tissue factor in the circulating blood with its synthesis initiated by the adhesion of monocytes to the activated endothelial cells. On the other hand, Day and co-authors (6) suggested that the main source of the tissue factor can be attributed to the vascular wall. Independently of the above-mentioned differences the authors of numerous publications came to the conclusion that the circulating tissue factor is connected with the presence of micro particles or micro-vesicles. Lopez and co-authors (3) postulated that the micro-vesicles containing the tissue factor are the key link in the pathogenesis of venous thrombosis. Monocytes are stimulated to create tissue factor particles and micro-vesicles on its surface.

When determining the pathogenesis of venous thrombosis the following question should be answered: what phenomena precede the beginning of thrombosis? Many authors investigating the mechanism of the above-mentioned came to the conclusion that the beginning of venous thrombosis is connected with the presence of venous inflammation, as a result of the influence of such pro-inflammatory cytokines as interleukin 6 (IL-6), interleukin 8 (IL-8), TNFα, and MCP-1. It was expected that the presence of elevated concentrations of the above-mentioned mediators would precede the diagnosis of venous thrombosis, enabling its prediction. However, conclusions obtained from experimental and clinical trials are ambiguous.
Van Aken and co-authors (7) determined IL-6, IL-8 and MCP-1 in 182 patients with recurrent venous thrombosis and 350 healthy volunteers. Increased IL-6 values were observed in 25.8% of patients with thrombosis, IL-8 in 21.5%, and MCP-1 in 24.1%. These results showed a characteristic elevation of inflammatory mediators in patients with venous thrombosis, and in vitro studies confirmed that these cytokines increased tissue factor expression in monocytes.

Viles-Gonzales and co-authors (8) also observed that the activation of the coagulation system combined with the presence of thrombin and fibrin deposition resulted from the inflammatory condition of the vascular wall. According to the authors, both mechanisms-blood coagulation and inflammatory reaction are connected. Poredos and Jezovnik (9) noted that venous wall inflammation is the major factor responsible for the initiation of venous thrombosis, due to the activation of leucocytes, and their adhesive interaction with endothelial cells.

The connection between the inflammatory reaction and activation of the coagulation system is well-known in case of patients diagnosed with disseminated intravascular coagulation syndrome. It seems that the mutual effect of the inflammatory reaction and activation of the coagulation system can also be limited to a fragment of the venous vessel. The presence of C-reactive protein values might be useful in the early detection of deep venous thrombosis. According to Fox and Kahn (10) and Bucek and co-authors (11) the determination of C-reactive protein levels proved useless, considering the confirmation or exclusion of venous thrombosis. According to Tsai and co-authors (12) CRP measurements considering 5 presented publications did not confirm the presence of venous thrombosis. Reiter and co-authors (13) demonstrated that the number of leucocytes and C-reactive protein levels were significantly elevated in case of 37 patients with venous thrombosis. Thrombogenesis and resulting vascular wall damage lead towards secondary inflammatory reactions, manifested by increased leucocyte and C-reactive protein values.

According to one of many hypotheses, primary venous wall inflammation can result from venous stasis and venous wall ischemia, especially of the endothelium covering the venous valve pockets. During everyday activities deep venous blood flow is possible thanks to the muscles of the lower extremities. During muscle relaxation the pressure in the deep veins does not exceed 10-12 mm Hg, being even lower in the recumbent position. Lower leg muscle contractions increase the pressure of venous sinuses and deep veins even up to 200 mm Hg, and these repeated contractions during walking reflect the oxygenation of the above-mentioned. Hamer and co-authors (14) demonstrated the partial oxygen pressure (pO₂) by means of an oxygenic electrode placed in the femoral veins of anesthetized dogs, and patients subjected to varicose surgery. The partial oxygen pressure was measured under the following conditions: presence of continuous linear venous flow, and pulsating flow imitating a venous-muscular pump. After 10 minutes of linear non-pulsating blood flow in patients and anesthetized dogs the partial oxygen pressure in the venous pockets was significantly lower, in comparison to the pressure measured in the axial blood flow. In four dogs, the above-mentioned measurements were performed 1.5 and 2 hours since the initiation of the non-physiological venous flow demonstrating that the partial oxygen pressure, considering the venous pockets was near zero. The venous valve cusps are deprived of their own vasa vasorum, and their oxygenation depends on the oxygen content of the circulating blood. Endothelial ischemia can lead towards venous valve pocket thrombosis. Venous stasis can lead towards hemoglobin desaturation and endothelial damage, due to ischemia, including the necrobiosis of endothelial cells (15).

Kearon (16) described the natural history of venous thrombosis demonstrating that thrombosis usually develops after surgical procedures in the deep veins of the lower legs, often originating from the venous recess. Ouriel and co-authors (17) investigated phlebographic data of 885 patients with lower extremity venous thrombosis, demonstrating that peripheral thrombosis was more frequent, as compared to proximal: in 83% of patients thrombosis was present in the deep veins of the lower legs, most often in case of the peroneal vein (67%). The frequency of deep vein thrombosis after surgical procedures without prophylaxis ranged between 36% and 49% (4 pts), considering patients on unfractionated heparin between 12% and 27%
(6 pts), in patients with elastic stockings between 11% and 23% (5 pts), and in 2-4% considering patients with elastic stockings and unfractionated heparin (4 pts). The relationship between deep venous lower extremity stasis and thrombosis poses no doubts. Thus, venous thrombosis prophylaxis seems an important issue.

Elastic stockings in case of patients with already existing venous thrombosis will not improve venous patency. Therapy consists in the administration of anticoagulation drugs. According to guidelines established by the American College of Chest Physicians (18) initial therapy of patients with deep vein thrombosis or pulmonary embolism (apart from those hemodynamically insufficient) consists in the subcutaneous administration of low molecular weight heparin (LMWH), fondaparinux, or intravenous infusion of unfractionated heparin. The dose of the latter depends on the kaolin-kephalin (APTT) time. The authors of the above-mentioned guidelines also suggested the administration of oral vitamin K antagonists (USA-warfarin, Poland-acenocumarol or warfin). All the above-mentioned inhibit the coagulation system: unfractionated heparin inhibits thrombin and factor X. Low molecular weight heparin inhibits factor X more effectively than thrombin, while fondaparinux bonds with antithrombin inactivating factor X more efficiently. Vitamin K antagonists inhibit the final carboxylation of four coagulation factors: II – prothrombin, VII – proconvertin, IX – factor Christmas, and X – factor Stuart, leading towards their hemostatic ineffectiveness. The assumption connected with heparin, pentasaccharide and vitamin K antagonist therapy consists in the inhibition of the activity of the coagulation system and progression of thrombosis.

Opinions concerning the influence of heparin on the venous vascular wall at the sight of thrombogenesis are infrequent. Downing and co-authors (19) evaluated the influence of unfractionated and low molecular weight heparins on the histological and morphological changes of the inferior vena cava of rats, subjected to vein ligation for a period of six hours, in order to obtain thrombosis. Every type of heparin was injected one hour before vein ligation. The Authors observed a significant decrease in the amount of morphometric venous wall inflammatory lesions in animals following heparin injection, including a reduction in the number of neutrophils penetrating the venous wall in case of animals receiving low molecular weight heparin. The authors suggested that LMWH might demonstrate anti-inflammatory activity, being different from its anticoagulative activity. According to Wakefield and co-authors (20) heparin sulphate, the most commonly used anticoagulation drug in patients with venous thrombosis demonstrates poor anti-inflammatory properties. Based on data observed by Dawes (21) heparin inhibits complement activity.

Tyrrell and co-authors (22) did not unequivocally show the anti-inflammatory activity of heparins.

It is assumed that the development of an inflammatory focus inside the venous vessel initiates thrombogenesis by means of leukocytes transferred via the blood stream to the inflammatory focus in the vascular wall. During the initial stage leukocytes are bound to the vascular wall and endothelium, which favors the interaction between selectin proteins and their glycoconjugated ligand. The vascular leukocytes come into contact with specific chemo-attractants, initiating the second stage of adhesion, where leucocyte integrins bond with their endothelial ligands. The leucocytes penetrate through the endothelium and migrate towards the inflammatory lesion.

Eppihimer and Schaub (23) demonstrated that adhesion and transmigration of leucocytes through the endothelium contribute to the initiation of venous thrombosis following the destruction of the endothelial cell barrier and exposure of the basilemma. During the initial stage of the above-mentioned process the key role is attributed to adhesive proteins, especially selectin. The pro-adhesive activity of P-selectins results from their reaction with glycoprotein ligand-1 (PSGL-1), present in most circulating leucocytes. The binding of P-selectin, present in the endothelium or platelets with PSGL-1 leads towards the following interactions: leucocyte – endothelial cell and leucocyte – platelet (24). The intention of the authors of experimental studies was to disrupt the bond between P-selectin and its ligand, and inhibit the inflammatory reaction inside the venous wall during its early stage. Wakefield and co-authors (25) observed significant inhibition of venous thrombosis and anti-inflammatory reactions, as well as reduction
considering the expression of pro-inflammatory cytokines (IL-8) in the venous wall of baboons. The animals received a recombinant P-selectin glycoprotein ligand (rPSGL-Ig) before the development of venous thrombosis. The above-mentioned ligand was responsible for P-selectin binding and prevented leucocyte inhibition, which in turn inhibited the inflammatory process at the site of venous insufficiency. The Authors concluded that the inhibition of the inflammatory process following the administration of rPSGL-Ig even without anticoagulation therapy leads towards effective prophylaxis of venous thrombosis. Similar results were obtained by the above-mentioned authors, and Downing and co-authors (26), who demonstrated that monoclonal antibodies against P-selectin reduce the incidence of venous thrombosis in baboons. The binding of P-selectin with the appropriate antibody has a beneficial effect on the inflammatory reaction of the venous wall.

Considering an experimental study undertaken on mice the authors determined the influence of an oral selectin-binding inhibitor (PSI-697) on the course of venous thrombosis following the ligation of the inferior caval vein (27). The administration of the above-mentioned inhibitor significantly reduced the weight of the thrombus without an anticoagulative effect. The authors concluded that this was the first evaluation of the new bioavailable P-selectin inhibitor (PSI-697). Considering another experimental animal study published in 2008 (28) the authors evaluated the prophylactic and therapeutic effect of the new oral P-selectin inhibitor—PSI-421. Considering the group of animals that received the above-mentioned the percentage of patent veins was higher, while the inflammatory reaction was lower, as compared to animals receiving enoxaparin, and the control group. Thus, study results exist showing the inhibition of venous thrombosis following the binding of P-selectin (early thrombosis adhesive protein), inhibiting the inflammatory reaction without interference in the coagulation system mechanism.

Downing and co-authors (29) demonstrated on rat models who were subjected to thrombosis following inferior caval vein ligation that the infusion of an anti-inflammatory cytokine (IL-10) not only reduced the venous inflammatory reaction, but also limited thrombosis itself.

The above-mentioned studies confirmed the inhibiting effect of anti-inflammatory substances, although were only limited to experimental investigations. Similar effects could be expected following the use of non-steroid anti-inflammatory drugs. Most non-steroid anti-inflammatory drugs exert a non-selective effect as cyclooxygenase inhibitors (COX), considering phospholipids metabolism. Phospholipase \( A_2 \) transforms phospholipids into arachidonic acid, which in turn is transformed by means of oxygenation into prostaglandin \( \text{H}_2 \) (PG\( \text{H}_2 \)) under the influence of cyclooxygenase isoforms: COX-1, COX-2 and COX-3. Prostaglandin (PG\( \text{H}_2 \)) is changed into prostanoids depending on the type of synthase-enzyme responsible for the transformation: prostaglandin \( \text{E}_2 \) (PG\( \text{E}_2 \)), prostacyclin (PG\( \text{I}_2 \)), and thromboxane (TX\( \text{A}_2 \)).

The influence of these prostanoids on the hemostatic mechanism is well-known: prostacyclin is synthesized in endothelial vascular cells and the smooth muscular layer, dilating vessels and showing platelet anti-aggregation activity. On the other hand, thromboxane is synthesized inside the platelets, its activity being reverse to that of prostacyclin: stimulates platelets towards aggregation and the smooth muscular layer towards contraction. Prostaglandin \( \text{E}_2 \) is released at the site of inflammation, and similarly to prostacyclin, is responsible for the dilation of vessels.

Most non-steroid anti-inflammatory drugs inhibit two cyclooxygenase isoforms: COX-1 and COX-2. COX-1 isoform inhibitors decrease the synthesis of thromboxane \( \text{A}_2 \) and prostacyclin. On the contrary, COX-2 isoform inhibitors have no negative effect on thromboxane \( \text{A}_2 \) synthesis, however, decrease the synthesis of prostacyclin. According to Grosser and co-authors (30) the COX-2 isoform is responsible for the transmission of pain, fever, and inflammation, while the COX-1 isoform is responsible for the activity of platelets.

Most non-steroid anti-inflammatory drugs act as non-selective cyclooxygenase inhibitors. Non-steroid anti-inflammatory drugs are used in the treatment of superficial thrombophlebitis, if the thrombus does not infiltrate into the deep veins. Therapy usually lasts for a period of one week under the form of intramuscular injections, in order to avoid digestive tract complications, and does not require anticoagulation drugs (31). Treatment of superficial
thrombophlebitis accompanied by thrombus infiltration into the deep venous system differs: anticoagulations drugs are required, in order to avoid pulmonary artery embolism.

The use of non-steroid anti-inflammatory drugs in case of deep venous thrombosis is not commonly accepted. Doutremepuich and co-authors (32) investigated the combined administration of LMWH (fraxiparin) and the non-steroid anti-inflammatory drug- phenylbutasone on the thrombogenesis, following inferior caval vein ligation in rats. The Authors demonstrated that the weight of the thrombus was significantly lower in rats receiving higher doses of fraxiparin (2 mg/kg), as well as in those receiving lower doses of fraxiparin (1 mg/kg), in addition to phenylbutasone (1 mg/kg). Moreover, the injection of phenylbutasone at a dose of 10 mg/kg reduced the weight of the thrombus, similarly to that observed after the injection of fraxiparin at a dose of 2 mg/kg. The combination of both antithrombotic drugs (low doses) exerts a better anticoagulative effect, as compared to fraxiparin and phenylbutasone administered separately in high doses.

In 1994, Nielsen and co-authors (33) published a study comparing results obtained following therapy of deep thrombophlebitis diagnosed by means of phlebography in 90 patients. Forty-eight received an intravenous infusion of unfractionated heparin, followed by an oral anticoagulant (Phenprocoumon) for a period of 3 months. The remaining 42 patients received oral phenylbutasone: the first day- 3x200 mg followed by 3x100 mg for nine days. All patients were mobilized since the day of admission, and wore low compression elastic stockings. Phlebography of the lower extremity veins was performed after admission and 30 days. Perfusion and ventilation isotopic lung examinations were performed within 48 hours of admission, and repeated after 10 and 60 days.

Repeated venous phlebography demonstrated no significant difference in the progression or regression of venous thrombosis, considering both patient groups. Isotopic lung examinations performed in all patients before treatment showed that 49% were diagnosed with asymptomatic pulmonary embolism, and control examinations demonstrated no differences in the course of the disease, independently of the group. Phenylbutasone therapy was complicated by heart failure, observed in five patients, requiring the administration of diuretics, although phenylbutasone was not discontinued.

The authors of the study (34) pointed to the fact that all patients were mobilized since the beginning of treatment, in addition to the use of elastic compression stockings. Early patient mobilization and compression stockings seem extremely important, since bedridden patients are burdened with the risk of thrombogenesis. The authors noted no difference between the course of the venous thromboembolic disease, considering patients treated by means of anticoagulation drugs or oral phenylbutasone for a period of ten days. Phenylbutasone was not considered as a non-steroid anti-inflammatory agent, but merely as an analgesic.

Wang and co-authors (34) evaluated the efficacy of another non-steroid anti-inflammatory drug – indomethacin – in the prophylaxis of deep thrombophlebitis, considering patients subjected to total knee joint replacement. One hundred and fifty patients were divided into three groups: group I patients- control group without prophylaxis, group II – patients receiving low molecular weight heparin (fraxiparin), and group III – patients receiving oral indomethacin at a dose of 25 mg, twice daily. Prophylaxis using fraxiparin or indomethacin was initiated the day before the operative procedure, and continued until hospital discharge. The percentage of patients with deep venous thrombosis amounted to 71% (group I), 50% (group II) and 45% (group III). According to the authors indomethacin inhibits the aggregation of platelets and reduces the level of thromboxane B2, in addition to being a potent postoperative analgesic (hospital in Taiwan). The authors observed a reduction in the frequency of postoperative venous thrombotic complications, considering patients receiving fraxiparin and indomethacin: 50% in both groups. The positive response to indomethacin was attributed to the inhibition of platelet aggregation and decreased level of thromboxane B2.

The aggregation of platelets necessary for proper hemostasis is also considered as the early stage of venous thrombogenesis. Knowledge of the above-mentioned enabled to administer acetylsalicylic acid (ASA) in the prevention of venous thrombosis. Absorbed by the digestive tract ASA infiltrates the platelets bonding with their cyclooxygenase, inactivat-
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The binding of ASA and COX-1, results in the permanent inhibition of thromboxane A₂, necessary for platelet aggregation. This can be obtained using small doses of ASA (50-100 mg). The inhibition of the COX-2 isoform, manifested by the anti-inflammatory and analgesic effect requires higher doses of ASA.

Clinical studies determining the efficacy of ASA in the prevention of thrombogenesis, compared the efficacy of ASA with another antithrombotic drug, or combined therapy of the two, as compared to yet another antithrombotic agent.

Flicoteaux and co-authors (36) subcutaneously injected 5000 units of unfractionated heparin 2 hours before, 12 hours after, and every 8 hours thereafter for a period of ten days following total hip replacement surgery. The second group of patients received similar doses of heparin, in addition to 500 mg of intravenous ASA given 2 hours before and 12 hours after the operation. After surgery these patients received the same dose of ASA every 12 hours for ten days. There were no group differences, considering postoperative deep venous thrombosis (20% of patients). However, the average intra- and postoperative blood loss was higher, considering patients receiving ASA and heparin (2273.90 ml), in comparison to those receiving heparin only (1796.95 ml).

Vinazzer and co-authors (37) divided patients subjected to surgery into three groups: group I patients received ASA 500 mg, twice daily (intravenously until day 3) followed by oral ASA for at least 7 days. Group II patients received the standard subcutaneous dose of 5000 units of unfractionated heparin every 12 hours-the first dose was administered the night before surgery and continued for at least 7 days. Group III patients received both drugs, according to the above-mentioned scheme. The frequency of postoperative venous thrombosis was significantly lower, considering patients receiving heparin and ASA, as compared to those receiving only heparin or ASA. However, hemorrhagic complications occurred more often in patients on both drugs.

In 1994, a randomized study was published (38), considering management inhibiting platelet aggregation, based on literature data obtained until March, 1990. The aim of the above-mentioned therapy was to prevent deep thrombophlebitis and pulmonary artery embolism in patients subjected to general and orthopedic surgery, or immobilized due to other reasons. The study group comprised 9000 patients. The Authors demonstrated that antiplatelet therapy lasting between 1 and 3 weeks significantly reduced the frequency of deep thrombophlebitis and pulmonary artery embolism in surgical patients. Several weeks of therapy can decrease the occurrence of thrombophlebitis by 50% and pulmonary embolism by 2/3. Benefits of such treatment seem more pronounced in case of high-risk patients-orthopedic procedures. Thus, results demonstrated that antiplatelet therapy, either by means of antiplatelet drugs or in combination with heparins should be considered during prophylaxis.

Their exist divergences between guidelines considering indications towards ASA therapy in the prophylaxis of deep thrombophlebitis and pulmonary embolism. The Scottish Intercollegiate Guidelines Network published in 2002 (39), recommendations considering the prevention of venous thromboembolic disease. The meta-analysis of 53 randomized clinical trials evaluating the use of antiplatelet drugs (ASA most often) in the prevention of venous thromboembolism, considering patients subjected to general or orthopedic surgery demonstrated the decrease of asymptomatic venous thrombosis from 35% to 26%, pulmonary embolism from 1.6% to 0.6%, and fatal embolism from 0.6% to 0.2%, as compared to the control group. The Authors of the above-mentioned guidelines recommend the use of ASA, as an effective prophylactic drug in patients subjected to surgical procedures demonstrating that ASA, similarly to heparin, is as effective in the reduction of fatal pulmonary embolism cases. These guidelines differ from those used in North America (40), not recommending ASA in the prophylaxis of venous thromboembolic disease.

The presented differences between antiplatelet drugs preventing the occurrence of venous thromboembolic disease are constant, considering both continents. Based on guidelines elaborated by the American College of Chest Physicians (2008) considering the prevention of venous thromboembolism, Geerts and co-authors (41) are against the use of ASA in case of the above-mentioned.
In 2002, Łopaciuk, Zawilska, Torbicki and co-authors (42) published guidelines, considering the prophylaxis and treatment of venous thromboembolism. The authors do not recommend the use of ASA, as the only method in the prevention of venous thromboembolism, considering patients subjected to orthopedic procedures, knowing that these procedures are burdened with the risk of the above-mentioned disease entity.

As previously mentioned the main clinical investigations concerning venous thrombosis were aimed at hemostasis system disturbances. Consequently, preventive and therapeutic methods of venous thrombosis were focused on the inhibition of the activity of the hemostatic system. The efficacy of these methods can be improved if we combine the inhibition of the hemostatic system with improvement of deep venous blood flow or suppression of the inflammatory reaction of the vascular wall.

Both Hamer and co-authors studies, as well as clinical observations justify the efficacy of improving deep venous lower extremity blood flow in the prevention of thrombosis. The easiest method improving blood flow is the use of elastic stockings: “knee-length”, since most venous thromboses originate at the level of the lower legs. The combination of elastic compression and pharmacological methods, such as subcutaneous injections of LMWH significantly reduce the number of venous thrombotic complications, especially after surgery. Due to the divergence of available data there is no justification as to the prophylaxis of venous thrombosis following the administration of acetylsalicylic acid (ASA).

It can be assumed that in the future treatment of thrombophlebitis will consist in the early administration of drugs inhibiting the activation of the coagulation system and the inflammatory reaction of the venous wall. The dilemma concerning the origin of venous thrombosis: thrombogenesis of the venous ependyma or inflammatory focus development will be in the near future a philosophical, rather than a therapeutic problem. According to current knowledge, both above-mentioned pathological processes should be subjected to early and energetic treatment.

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