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6 Immune-mediated coagulation disorders in cancer patients

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Abstract

Cancer patients express a diversity of coagulation disorders, sometimes as a first sign of malignancy. The incidence of subsequent occult cancer is 13% in the group of patients with symptomatic idiopathic venous thromboembolism. Procoagulative activity results from neoangiogenesis related to the tumour growth, and tumour induced extrinsic coagulation pathway activation. It is characterised with increased production of fibrinogen and other coagulation cascade components, followed by enhanced fibrinolytic system activity. An increased platelet activation and turnover are commonly observed. A liver dysfunction

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due to adjuvant chemotherapy, radiotherapy, postoperative hypoproteinemia, and defective myelopoiesis in several neoplastic processes may further pronounce coagulation disbalance, resulting in hypercoagulability and/or bleeding.

The association between autoimmune coagulation disorders and neoplastic diseases, especially lymphoproliferative neoplasm is well known. Elevated antiphospholipid antibodies levels, a reliable marker of cancer activity, were found in 40 % of patients with non-Hodgkin's lymphoma at diagnosis. Autoimmune haemolytic anaemia, immune thrombocytopenia, thrombotic thrombocytopenic purpura and lupus anticoagulant may be characteristic complications in patients with malignancies. These are somewhat different than other cancer related coagulopathies, usually correlating with poor survival. A successful treatment or surgical removal of underlying malignancy may result in improvement of coagulation disorder. This article discusses biochemical base, clinical presentation, diagnostics, and complications of immunologically linked coagulation disorders in cancer patients.

Introduction

Patients with malignancies are known to suffer more coagulation disorders compared to general population, sometimes as the first sign of neoplastic disease [1, 2]. In the population of apparently cancer free patients with idiopathic venous thromboembolism, occult cancer was found in 13 % [3]. Autoimmune diseases are more frequently observed in this cohort, too. Pacini et al. had found positive thyroglobulin antibodies and/or microsomal antibodies in 23 % of patients with thyroid cancer [4], whereas Giani et al. observed 15 % incidence of autoimmune thyroid diseases in breast cancer patients [5].

Autoimmune coagulation disorders were found in patients with benign neoplasms like myxoma [6], and in the variety of solid or haematological malignant diseases i.e. splenic marginal zone B-cell lymphoma [7], acute lymphatic leukaemia (ALL) [8], and breast cancer. Autoimmune coagulation disorders like haemolytic anaemia, immune thrombocytopenia, acquired bleeding disorders and positive Coomb's test may be observed in 20 % of these patients [9]. Since disease progression is significantly more frequent in patients presenting an immunological event, these disorders may have prognostic value [9, 10]. After cancer is successfully removed or treated using chemotherapy [11], coagulation disorder usually stabilizes.

The important difference between coagulation disorder in cancer patients and in general population is a triggering factor. Thrombophilias or bleeding disorders in general population are usually linked to deficiencies or mutation

in the genes encoding coagulation cascade enzymes and are triggered by stimulants like trauma, surgical procedures, pregnancy, puerperium or oral contraceptives. These disorders can usually be confirmed by genetic sequencing in addition to standard coagulation tests. A coagulation trigger in malignancy is usually linked to highly immunogenic phospholipid components of tumour cell membrane or to proteins secreted by malignant cells. The presence of these substances can be confirmed by series of enzyme-linked immunosorbent assays to measure the concentrations of tumour antigens, and using coagulation tests to measure enzyme activities [10].

This article presents some most important autoimmune coagulation disorders in cancer patients and their pathophysiological basis.

1. Cancer growth and angiogenesis

While growing, malignant tumours usually produce intratumoural proteases [12] involved in the tumour cell invasion, numerous stromal reactions, and new blood vessel formation or angiogenesis [13]. A membrane type 1 matrix metalloproteinase (MT1-MMP) exposed at abluminal endothelial cell surface. MT1-MMP is enzyme engaged in direct fibrinolytic activity, allowing a destruction of basal membrane, which is essential for tubulogenesis of novel capillaries and tumour growth [14].

Tumour cells release stimulatory factors, like vascular endothelial growth factor (VEGF) and vascular permeability factor (VPF), which are indirect procoagulants, altering haemostatic properties of endothelial cells, and playing a key role in the pathophysiology of several tumours [15]. VEGF, VPF and MT1-MMP are basic regulators of tumour-induced angiogenesis and growth, recently recognised as potential targets for antitumour immunotherapy. Inhibitors of VEGF receptor-1 and –2 as well as angiogenesis inhibitors are new modalities of anticancer chemotherapy [16]. Those may become the safest and least toxic of anti-cancer therapies. Anti-VEGF antibody bevacizumab, recently approved by FDA, is in the focus of the scientific community, alone or combined with other cytostatics [17, 18].

The vasculature in the tumour microenvironment presents multiple structural and functional abnormalities, both in endothelium and in the vascular basement membrane. The inner coat is defective, not fully endothelial-lined, lacking especially in aggressive tumours. The basement membrane in tumour vessels has conspicuous abnormalities, including irregular thickness, multiple layers, and loose association with endothelial cells and pericytes [19]. A leakage of plasma protein through highly permeable tumour blood vessels may result in the tumour-induced extrinsic pathway of coagulation. A direct consequence is abundant expression of fibrinogen

indicative of leakage of plasma proteins in the extracellular matrix of the tumours [15] as well as in blood of cancer patients.

Furthermore, during the tumour growth a diffuse bleeding into various organs may result from severe damage of the capillary membrane. Immunoglobulin G (IgG) λ-subtype myeloma derived paraprotein may accumulate in the dermal matrix resulting in haemorrhagic-bullous eruptions. Immunohistochemistry may reveal dense pericapillary and perivascular deposits of IgG indicating a paraprotein mediated damage of the capillary membrane [20]. An immunoglobulin A (IgA) paraprotein secreting myeloma was described to be the underlying cause of pulmonary hypertension, presenting with diffuse alveolar haemorrhage [21, 22]. The diffuse bleeding usually stops after initiation of treatment consisting of anticancer chemotherapeutics and corticosteroids [21, 22].

1.1. The changes in the membrane architecture

The structural changes in the membrane architecture are typical in rapidly growing cancer cells. These may result from increased cell proliferation, lack of nutrients and subsequent increased apoptosis rate observed during initial neoplastic transformation [23]. Cells undergoing apoptosis have a typical morphologic disturbance: phosphatidylserine (PS) from the inner side of cell membrane exposes at outer cell surface [24]. Since PS is highly immunogenic substance it promotes both chemotactic and immunological reactions, and plays an important role in immunological coagulation disorders [25]. A direct involvement of PS in coagulation activation was demonstrated in non-malignant cells like monocytes [25], activated platelets [26], and malignant cells in non-Hodgkin lymphoma [27].

Fernandes and co-workers demonstrated highly procoagulant properties, leading to robust thrombin formation of C6 glioma cells. This reaction results from concomitant tissue factor (TF) exposure and from the presence of the anionic lipid – phosphatidylserine at the outer leaflet of the cell membrane. An extracellular protein, annexin V, which blocks PS binding sites, inhibited factor X (FX) and prothrombin conversion into active enzyme forms by their respective C6-assembled activating complexes. PS-containing vesicles achieved the same procoagulant effect as cells expressing PS on the cell membrane [23]. Antibodies against PS were detected in the majority of neoplasms [26, 27].

Due to the involvement in the coagulation activation, exposure of phosphatidylserine on the outer side of cancer cells membrane may become useful in the antitumour therapy [28]. The development of a liposome formulation containing PS may be of potential therapeutic utility if these can be designed to achieve tumour selective thrombosis. Chiu et al. [29] have evaluated binding of the vascular cell adhesion molecule 1 (anti-VCAM-1)

Antibody-conjugated PS liposomes to VCAM-1 using two *in vitro* models, and assessed the ability of these liposomes to catalyze blood coagulation reactions. Authors have found that the liposomes were capable of catalyzing blood coagulation reactions upon the exposure of the thrombogenic PS membrane surface [29]. Dienst at al. observed convincing tumour tissue necrosis and intratumour vascular fibrin thrombosis following a single systemic administration of recombinant fusion proteins consisting of soluble tissue factor (sTF) fused to antibody fragments directed against mouse or human VCAM-1. In addition, long-term treatment induced tumour-selective intravascular coagulation, tumour tissue necrosis, and statistically significant tumour growth delay [30]. Such selective effect targeting thrombogenesis in tumour vasculature is a matter of ongoing studies and demands more further investigations.

A correlation of changes in the membrane structure and autoimmune coagulation disorders was indirectly pointed to by the results of the retrospective analysis conducted by Barcellini and coworkers from Chronic Lymphocytic Leukaemia Group (GIMEMA) [31]. In a group of 194 B-cell chronic lymphocytic leukaemia patients with autoimmune diseases, the authors have found that the most frequent form of autoimmune complication was autoimmune haemolytic anaemia, (129 cases, or 66 %), followed by autoimmune thrombocytopenia (35 cases, or 18 %), whereas 30 patients have other autoimmune diseases (16%). Multivariate analysis confirmed that variables more associated with autoimmune complications were older age (OR 3.43), and as regards chemotherapy first line therapy (OR 15.62) and second line therapy (OR 48.64) were confirmed as risk factors. Stage C (disease progression) was confirmed as independent factor significantly related to the occurrence of autoimmune disease (OR 3.78) [31]. These results can be attributed to the accumulation of malignant B-CLL antigen-presenting cells [31], but also to the higher number of apoptotic cells presenting PS on the cell surface [24] in the advanced disease stage and after chemotherapy was started.

2. Antiphospholipid syndrome

The presence of the antiphospholipid antibodies (APA) is related to thrombotic events represented as an antiphospholipid syndrome with a syndrome of venous and arterial thrombosis, recurrent foetal loss and thrombocytopenia in a considerable proportion of carriers. The antiphospholipid syndrome is commonly observed in non-malignant and malignant diseases, like in myxoma [6], B-cell lymphoma [7], or in solid neoplasms [32]. Yoon et al. observed high incidence of APA (60.6 %) in cancer patients with thromboses [33]. It is supposed that phosphatidylserine triggers interleukin-6 production, and start immunological reaction leading to the primary APS [6]. APAs are elevated in

41 % of non-Hodgkin lymphoma patients (NHL) at diagnosis in aggressive NHL. Their level may serve as an independent prognostic variable of shortened survival [10, 33]. The most prevalent APA were anti-ß2-glicophorin I (GPI) IgA antibodies, which were present in 46.9 % of cancer patients with thromboses [33], and in 22.1 % of patients with non-Hodgkin's lymphoma [10]. Anti-ß2-GPI IgM were found in 16 % of NHL patients, whereas anti-ß2-GPI IgG were observed rarely [10]. APA in malignant diseases may promote demyelinating diseases in cancer patients [7].

Anticardiolipin antibodies (aCL Ab) are another class of antiphospholipid antibodies IgA, IgM, and rarely IgG [10]. There are several reports on increased levels of aCL antibodies in patients with malignant diseases with thromboses i.e. cholangiocarcinoma [34]. Coagulation tests showed elevated levels of fibrinogen, fibrinogen degradation product, D-dimer, and IgM aCL Ab [34]. Although aCL Ab were found in diverse neoplastic diseases they were not related to increased thrombosis risk in Genevresse's study on 90 patients [35] and Bairey's study on 86 patients [10].

Lupus anticoagulant (LA, i.e. prothrombinase complex) is an antiphospholipid antibody that prevents blood clotting in the test systems, although a person having a positive LA test may have high thrombosis risk [7]. It is detected mostly through prolonged activated partial thromboplastin time (APTT). It is common in patients with malignant diseases, usually associated with other coagulation abnormalities [7, 36].

In clinical settings, the presence and not the titres, of APA were indicative for a subset of cancer patients with a high risk of developing thrombotic complications [32]. The study of Miesbach confirmed that among the group with solid tumours almost half of the patients (46%) had thromboembolic complications as an effect of underlying antiphospholipid syndrome [32]. Among the patients with haematologic and lymphoproliferative malignancies, 32 % suffered some thromboembolic complications. Venous thromboses were observed in 87.9 % and arterial in 24.2 % in this group, and were related to advanced cancer stage [33]. Thromboses can affect deep leg veins, peripheral veins, manifesting as microinfarctions, or abdominal veins resulting in hepatic veno-occlusive disease, hepatic infarction, cirrhosis, portal hypertension, autoimmune hepatitis, biliary cirrhosis, acute intestinal infarction, and intestinal bleeding, splenic infarction and acute pancreatitis [37]. APA can be detected by clotting assay or by enzyme-linked immunosorbent assay according to the Sapporo criteria [37].

3. Inhibitors of circulating coagulation factors

The blood coagulation cascade consists primarily of proteins, a series of enzymes or cofactors, which in turn, upon their activation lead to thrombus formation and intravascular coagulation (Fig. 1). The function of proteins

involved in blood coagulation is shown in Table 1. All of these proteins are present in the plasma as zymogens which are activated by cleavage of the polypeptide chain. Autoantibodies directed against clotting factors, or coagulation inhibitors, can induce life-threatening bleeding with a mortality rate up to 22%. One patient may present more coagulation abnormalities at the same time, and on the other hand, one antibody may inhibit activity of several coagulation factors. Multiple myeloma patients showed elevated Factor VIII, activated protein C (APC) resistance and significantly reduced protein S activity [38].

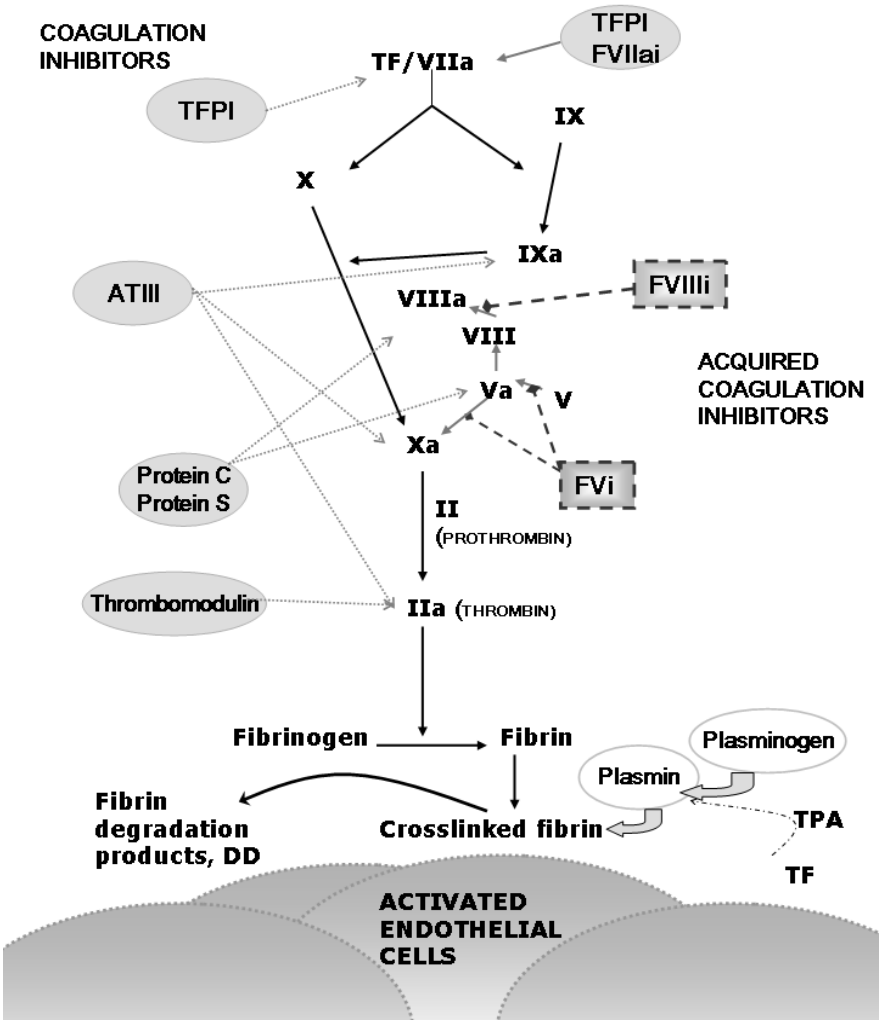

Figure 1. Coagulation cascade. A series of coagulation factors upon their activation lead to the fibrin cross-linking and formation of fibrin degradation products. Natural coagulation inhibitors (shaded oval forms) efficiently balance coagulation cascade and prevent the thromboses. Acquired coagulation inhibitors (shaded squares) observed in the cancer and in the variety of autoimmune processes may pronounce a bleeding diathesis.

Table 1. Proteins involved in blood coagulation and their functions.

Factor	Function
Coagulation Factors	
I (Fibrinogen)	Cleaved by thrombin to form fibrin clot
II (Prothrombin)	Serine protease activated on surface of activated platelets by prothrombinase complex (Ca^{2+}, factors Va and Xa)
III (Tissue factor)	A glycoprotein expressed on the surface of injured or stimulated endothelial cells to act as a receptor or cofactor for factor VIIa.
IV (Ca^{2+})	Acts as a cofactor.
V (Proaccelerin, labile factor)	Cofactor activated by thrombin. Factor Va is a cofactor in the activation of prothrombin by factor Xa.
VII (Proconvertin, serum prothrombin conversion accelerator)	Serine protease. Activated thrombin in the presence of Ca^{2+}.
VIII (Antihemophilia factor A)	Cofactor activated by thrombin. Factor VIIIa is a cofactor in the activation of factor X by factor IXa.
IX (Antihemophilia factor B, Christmas factor)	Serine protease activated by factor XIa in a presence of Ca^{2+}.
X (Stuart-Prower factor)	Serine protease activated on surface of activated platelets by tenase complex (Ca^{2+}, factors VIIIa and IXa) and by factor VIIa in presence of tissue factor and Ca^{2+}.
XI (Plasma thromboplastin antecedent)	Serine protease activated by factor XIIa.
XII (Hageman factor)	Serine protease activated by high-molecular weight kininogen and kallikrein. Binds to negatively charged surface at site of vessel wall injury.
XIII (Fibrin stabilizing factor, fibrinoligase)	Ca^{2+} dependent transglutaminase activated by thrombin in presence of Ca^{2+}. Stabilizes fibrin clot by covalent cross-linking.
Regulatory proteins	
Thrombomodulin	Endothelial cell receptor. Binds thrombin which then activates protein C.
Protein C	Cofactor of a serine protease. Activated by thrombin bound to thrombomodulin.
Protein S	Cofactor of protein C.

3.1 Acquired coagulation factor V inhibitor

Acquired inhibitors to factor V are considered rare events. Clinical features and the apparent associated risk of bleeding complications generally varied. The probable cause leading to the development of the inhibitors in cancer patients may be paraproteinaemia observed in malignant diseases, exposure to aminoglycoside antibiotics [39], surgery, as well as high levels of aCL Ab. Since inhibitor interference may exist, many of the cases also showed

evident co-associated lupus anticoagulant activity [40]. Other factors such as FII, FVII, FVIII, FIX, FX and FXI can be decreased, prothrombin time (PT) and activated partial thromboplastin time (APTT) prolonged, whereas thrombin time (TT) may show normal values [39]. Moderate thrombocytopenia and pathologic response to anticoagulant therapy can be also observed.

The inhibitor from IgG aCL class inhibits the activity of prothrombinase assembled from purified factor Xa and factor Va (Fig. 1), calcium ion, and phospholipids vesicles. It partially inhibits prothrombinase assembled from purified factor Xa, calcium ion, and normal platelets. The platelet factor V was relatively inaccessible to the antibody as proved by Nesheim et al. [39].

The diagnosis can be confirmed by inhibitor isolation by sequential affinity chromatography on protein A-Sepharose and factor V-Sepharose [39]. Other tests that can be used are Bethesda inhibitor assay, LA testing by the dRVVT (Russell's viper venom test) and kaolin clotting time (KCT). Residual factor levels can be detected by standard factor assays but using NPP diluted 1: 1 with buffer to define the 100% residual factor level [39].

3.2 Activated protein C (APC) resistance

A tissue-factor-based activated protein C (APC) resistance in the absence of factor V Leiden mutation is a disturbance of the haemostatic balance observed in malignant diseases. The acquired APC resistance is a common finding in T-cell prolymphocytic leukaemia and may significantly contribute to the cancer-related hypercoagulability [41]. It was present in 10-25 % of newly diagnosed multiple myeloma patients and significantly increased the risk of DVT and PE [38, 42]. Malignancy-associated thrombophilic state and paraprotein-specific mechanisms can explain the high rate of thrombosis in this cancer population. The treatment-induced changes observed in patients receiving chemotherapy with thalidomide potentiate risks of this transitional condition related to myeloma status [38]. Jiménez-Zepeda and co-workers observed deep venous thrombosis in 16 % of patients who received thalidomide at a median dose of 200 mg/qd. To minimize risks of combined chemotherapy authors suggest that effective prophylactic anticoagulation should be implemented at least during the first few cycles of treatment [43]. This malignancy related disorder was observed in solid tumours like metastatic breast and ovarian cancer. The laboratory investigation will confirm increased sensitivity to APC (>2.02 vs. 1.0 in healthy subjects), and commonly significantly elevated protein S levels, DD level, thrombin and fibrin formation compared with patients without metastases and healthy control subjects [44, 45].

3.3 Acquired haemophilia A

Acquired haemophilia A (AHA) is a coagulation disorder characterised by the spontaneous and iatrogenic bleeding and formation of neutralizing

antibodies (inhibitors) to Factor VIII in patients without a history of bleeding diathesis [46]. The most common bleeding types were haematuria, gastrointestinal bleeding and soft tissue haematomas [46]. Nearly 10 % of all patients presenting with AHA were proven to have an underlying solid or haematological malignancy [47]. The appearance of a Factor VIII inhibitor (Figure 1.) may be related to an altered immune status, as suggested by the fact that lymphoproliferative disorders (chronic lymphocytic leukemia, non-Hodgkin lymphoma, multiple myeloma, Waldenstrőm macroglobulinemia) are haematologic malignancies commonly associated with the development of inhibitors [48, 49]. Factor VIII inhibitor related to malignancies may arise due to the anticancer chemotherapy applied. English et al. reported FVIII inhibitor in a patient with chronic myelogenous leukaemia who developed significant bleeding and bruising at the site of bone marrow aspiration during the course of the interferon-α administration [49].

Antibodies to factor VIII may be considered as para-neoplastic phenomena among patients with a variety of solid tumours i.e. prostate and lung cancer [46, 50]. Although inhibitors to FVIII are usually of the IgG variety (IgG1 and IgG2), IgA and IgM monoclonal antibodies have also been described in lymphoproliferative malignancies, particularly in multiple myeloma and chronic lymphocytic leukaemia [48]. A location of the ligand-binding sites that are targets for inhibitory antibodies is shown in Figure 2.

Typical treatment suggested by modified Bonn Malmo protocol consists of (a) immunoadsorption for antibody elimination, (b) activated factor VIII substitution, (c) intravenous immunoglobulin substitution, and (d) immunosuppression [48]. A response rate for complete remission (CR) using the modified Bonn Malmo protocol was 91 % in overall population with FVIII inhibitor and 97 % when cancer patients were excluded [51]. In cancer patients with inhibitors against FVIII such therapy should be highly individualized on the basis of the patient's age, the titre of the inhibitor, the severity of the haemorrhage as well as the tumour type [48].

The treatment of cancer with chemotherapy or surgery will accelerate the eradication of inhibitors in most of patients [46, 50]. Sallah et al. reported 70 % of complete responses of AHA to cancer treatment [46]. A complete resolution of the circulating anticoagulant and a higher overall survival was more likely achieved in patients who had lower mean autoantibody titres and early-stage tumours than in those who had a persistently high titre of inhibitor. The presence of an underlying cancer is not a contraindication to the use of immunosuppressive therapy aimed at eliminating the autoantibody if the primary antitumour therapy has not eradicated the inhibitor. These patients should be treated in the same manner as other patients with AHA [48].

If factor VIII inhibitor appears during chemotherapy course, it should be discontinued. In patient with CML, a complete disappearance of the inhibitor

was described after interferon-α was discontinued. A therapy with the activated Factor VIII concentrates and prednisone was efficient and the patient did not have any further bleeding problems [49].

The diagnosis can be confirmed by isolated prolongation of the APTT, not corrected by incubating the patient's plasma with equal volumes of normal plasma (mixing study), associated with reduced Factor VIII level. Polyclonal auto antibodies belonging to an IgG4 subclass, monoclonal Riga or IBM antibodies can be by detected by immunoelectrophoresis [46, 48, and 50].

Since FVIII and VWF act as cofactors, their activity and concentrations are assessed through series of assays. There are five parameters that confirm Factor VIII and von Willebrand factor plasma concentrations and activity: Factor VIII: coagulant activity (FVIII: C); FVIII: antigen (FVIII: Ag), von Willebrand factor: antigen (VWF: Ag), von Willebrand factor: ristocetin cofactor (VWF: RCo), and von Willebrand factor: collagen binding (VWF: CB) [52].

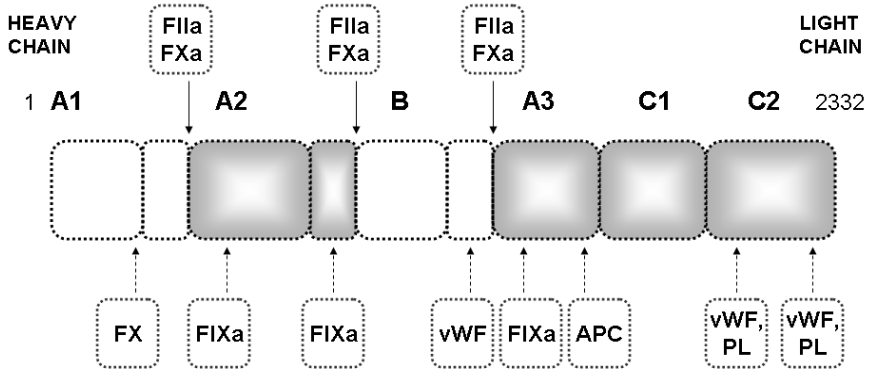

Figure 2. Structure–function relationship of factor VIII molecule and main epitopes of inhibitory autoantibodies. The circulating FVIII molecule is a heterodimer consisting of a heavy chain (domains A1, A2, and B) and a light chain (domains A3, C1, and C2). FVIII in plasma is non-covalently bound to von Willebrand factor (VWF) which protects it from inactivation by activated protein C (APC) [48]. The regions involved in binding to VWF are within the light chain preceding the A3 domain. The acidic regions (A) 1 and 2 and the binding sites for the FX, FIXa, and phospholipid (PL) membrane are also shown. Factor VIII is activated by thrombin and FXa, which cleave the FVIII molecule within the heavy chain and at the light chain. Inhibitors interfere with FVIII activity by preventing the interaction with FIXa, FX, PL, and VWF (the ligand-binding sites that are targets for inhibitory antibodies are shown in grey) or the thrombin cleavage. Modified from ref No. 48.

3.4 von Willebrand factor (VWF)

The von Willebrand factor (VWF) is a large glycoprotein stored in the Weibel-Palade bodies of endothelial cells and megakaryocytes [53]. In the response to vascular injury a rapid exocytosis of VWF occurs, which is essential for platelet aggregation and adhesion to the subendothelial matrix.

Levels of VWF in malignant diseases may be differentially affected. Enhanced VWF level is associated with tumour-related angiogenesis and may become a critical point in the haematogenous tumour cell metastasis [54]. Compared to their normal counterparts, increased synthesis of VWF was observed in various types of cancer, such as squamous cell larynx carcinoma, cervix carcinoma, colorectal carcinoma [55], as well as in metastatic osteosarcoma tumour samples, suggesting endothelial cells activation [56]. The ristocetin co-factor activity rose and increased highly polymeric plasma VWF antigen (VWF:Ag) in patients with disseminated metastases correlate with tumour progression and could be significantly higher in comparison with levels in healthy female controls and in women with benign breast disease and [57, 58].

The level of VWF in malignant patients is even higher due to deficient activity of specific metalloprotease, von *Willebrand factor-cleaving protease* (VWF-cp, ADAMTS13). The metalloprotease ADAMTS13 is synthesized mainly in the liver and cleaves ultralarge thrombogenic multimers of VWF derived from activated endothelial cells (Fig. 3). An inhibitor of ADAMTS13 leading to its deficient activity was shown to be IgG [58]. As a result of low ADAMTS13 activity in patients with colorectal cancer and advanced stage malignant tumours, an increase in the expression of uncleaved VWF can be observed [59]. A clinical disorder usually presents in the form of a syndrome called thrombotic thrombocytopenic. The syndrome is characterized by thrombocytopenia, haemolytic anaemia, fever, renal abnormalities, and neurological disturbances [60, 61].

The extent of the degradation of VWf by ADAMTS13 can be assessed using electrophoresis in sodium dodecyl sulphate–agarose gels and by immunoblotting. To determine whether an inhibitor of is present, protease activity in normal plasma can be measured after the incubation with patient's plasma [60]. Other determining methods are two-site immuno- radiometric assay, or VWF multimer analysis, residual collagen binding, residual ristocetin cofactor activity measurement and enzyme immunoassay [60, 62].

A deficiency of VWF may pronounce a bleeding disorder, *acquired von Willebrand syndrome*. In cancer patients this syndrome may be related to autoantibodies to the VWf, adsorption of VWF onto tumour cells or activated platelets, increase of VWF proteolysis and mechanical destruction of VWF under high shear stress [63]. It was observed in the multiple myeloma patients, patients with thyroid cancer, and in a variety of other malignancies [63, 64]. In the study of Mohri et al. inhibitor was identified as an antibody of the IgG class (IgG-λ Bence-Jones protein positive). It blocks the interaction of VWF with adhesion protein glycoprotein Ib (GPlb) in the presence of ristocetin and the binding of VWF to immobilized collagen type I, reacting with the epitopes on the A1 loop and A3 domains of VWF, but not with botrocetin-mediated interaction of VWF with GPIb [65]. The diagnosis can be confirmed mainly by

a decrease of ristocetin cofactor activity (VWF:RCo) and/or collagen binding activity (VWF:CBA) and by VWF multimeric analysis, usually with a selective loss of large multimers [63].

In the population with neoplastic diseases, a variety of other inhibitors can be also observed. The presence of inhibitors usually manifests with bleeding, prolonged PT and APTT, as observed in patient with non-Hodgkin's lymphoma and inhibitors against factor IX associated with AP As [27]. Another acquired prothrombin (Factor II) deficiency resulting from noninhibitory antibody to prothrombin that interacted with a calcium dependent epitope was found in a patient with a low grade lymphoma [66]. These severe bleeding complications completely disappeared, and coagulation parameters normalized after the chemotherapy of malignant disease was completed.

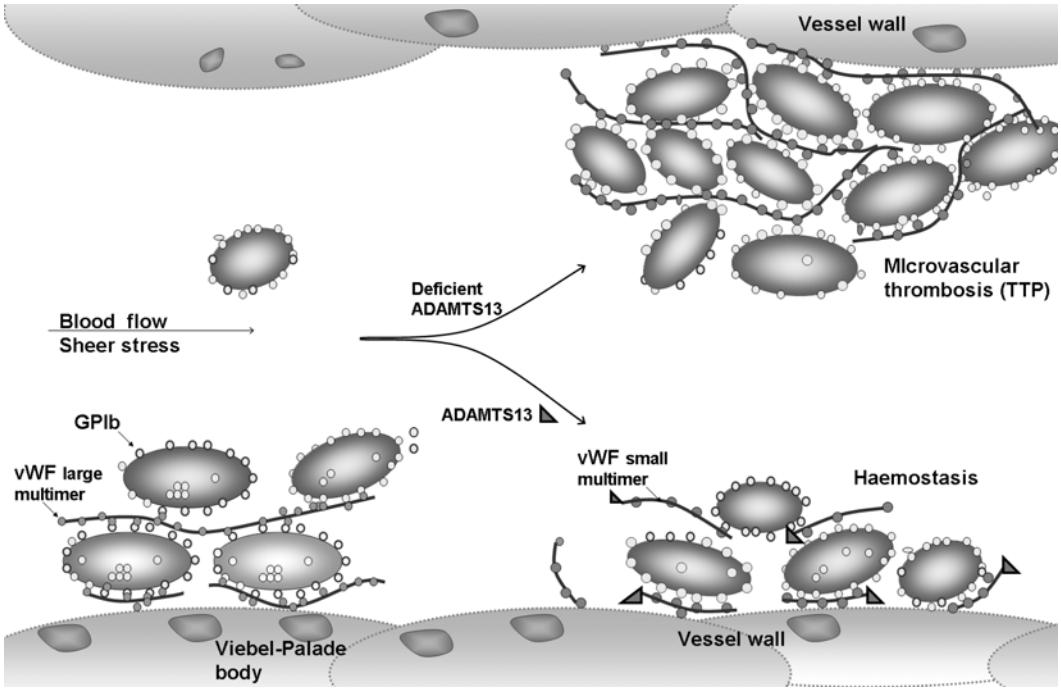

Figure 3. Unusually large von Willebrand factor (ULVWF) multimers are secreted in long strings by Viebel-Palade bodies from activated endothelial cells. The ULVWF multimer may be anchored in the endothelial cell membrane by P-selectin molecule. Under normal flow conditions ULVWF is cleaved by the metalloprotease ADAMTS13 into smaller VWF subunits, which circulate and do not induce adhesion and platelet aggregation. If coagulation cascade is triggered, these smaller multimers bind to GPIb on platelet surface and on the subendothelial collagen of damaged endothelial cells promoting thus normal haemostasis. In the absence, or functional deficiency of ADAMTS13, uncleaved ULVWF multimers induce platelet aggregation and adhesion to the endothelial cells leading to the microvascular thrombosis. The ULVWF multimers and large platelet aggregates may detach from endothelial cells and embolise distal microvessels as observed in the TTP [61].

4. Immunological platelet disorders

Platelet disorders are generally classified as quantitative, in which the platelet number is altered and usually decreased, or as qualitative disorders, in which the platelet function is affected. Thrombocytopenia is the most common platelet disorder in cancer patients, resulting from platelet consumption in morphologically imperfect tumour blood vessels [19]. A collagen from the basement membrane reaches blood stream through multiple holes in the endothelium, and activates platelets within the tumour vessels. Activated platelets express phosphatidylserine on the membrane and promote coagulation cascade [26]. Upon their activation platelets release growth factors and associate with fibrinogen in aggregates, resulting in elevated fibrinogen degradation products (FDP) levels in cancer diseases. Consequently, an increased platelet turnover in the cancer patients was observed [15]. Kuenen et al. observed that 40 % of patients with malignancy (in small series) had an elevated platelet count [16]. With the increased tumour size, the platelet production becomes inefficient vs. consumption, so that platelet count decreases. The lower preoperative platelet count thus correlates with inferior and the higher with superior survival outcome being indirect prognostic factor, like observed by Schwarz et al. in patients with periampullary adenocarcinoma [67]. Thrombocytopenia in cancer patients is commonly a non-immunologic consequence of myelosupression due to the chemotherapy. A smaller number of patients will develop immunological disorder, before initiation of cancer treatment or after antitumour chemotherapy was started [68]. Treatment induced thrombocytopeniae both nonimmune [69] and immune [68], are new clinical entity that may appear as a sole disease or associated with haemolytic anaemia [68, 69], and even pancytopenia [70].

4.1 Autoimmune thrombocytopenia

Autoimmune thrombocytopenia (AITP) is a decrease in the platelet count mediated by the presence of specific antibodies against platelets [31]. This autoimmune disorder was observed alone or as a part of immune pancytopenia [70]. Barcellini et al. have found AITP in 18 % of patients with MM having autoimmune disorders [31]. The AITP was described in splenic marginal zone cell lymphoma (SMZCL) [71], in NHL in the immuno-compromised patients with chronic granulocytic leukaemia after haematopoietic stem cell transplantation [72] as well as in solid tumours like thymoma or colorectal carcinoma [69, 70, 71, 73], Magagnoli et al. attributed an occurrence of AITP to the activity of the underlying lymphoma, recognizing it as a first manifestation of the active disease that allows the emergence of clones having autoantibody activity [71]. According to this hypothesis, the AITP can be interpreted as a neoplastic phenomenon, potentially curable by specific

therapy. In lymphoproliferative diseases, the AITP was supposed to be a consequence of the chronic immune system stimulation, which may, apart of persistent malignancy, be additionally triggered by *Helicobacter pylori* or by hepatitis viral infection [71]. There are several recent reports on immune thrombocytopenia triggered by oxaliplatin therapy [68]. Clinical features of the disease are petechiae, gastrointestinal bleeding, haematuria, acute renal failure, jaundice, mental disorder, preceded by thrombocytopenia and subsequent anaemia. A thrombotic form of the disease, thrombotic thrombocytopenic purpura (TTP) is accompanied by microangiopathic haemolytic anaemia [73].

The diagnosis can be confirmed by a fact that anti-platelet IgG on platelets from patients with idiopathic thrombocytopenic purpura (ITP) is greater than in normal people, as determined by anti-platelet antibodies directly on the platelet surface with a quantitative complement lysis-inhibition-assay. The platelet-associated IgG (PAIgG) may be assumed as evidence of ITP, although is not specific autoantibody for ITP because it increases in other than immune ITP patients [74]. PAIgG is presently measured by the competitive micro ELISA and flow cytometry methods [74]. Although the utility of analyzing PAIgG using flow cytometry is not considered standard for ITP diagnosis according to the policy of the American Society of Hematology, a study of Nishioka et al. suggests that flow cytometry is also useful for ITP screening [74]. In the drug induced ITP nonreactive red blood cell eluates, platelet-bound antibodies to GPIIb-IIIa, GPIb-IX, and GPIa-IIa with oxaliplatin-dependent antibodies and platelet autoantibodies can be found with standard techniques. Serologic findings will confirm a positive immunoglobulin G direct antiglobulin test [70].

Qualitative or functional acquired platelet disorders are occasionally reported in patients with malignant diseases. Tumour induced platelet aggregation is typically enhanced in various malignant diseases. On the opposite, it may be decreased in cancer patients in the presence of antibodies to platelet tumour adhesive molecules. Platelet-tumour aggregation could be completely inhibited when both tumour cells and platelets were incubated with monoclonal antibodies directed against the VWf binding epitope of GPIb-α and against the GPIb binding epitope of plasma VWf, respectively. Antibodies to VWf-cleaving protease may result in further modulation in the adhesion process and may reduce primary platelet-tumour adhesive interactions involved in the metastatic process [58].

Acquired Glanzmann's thrombasthenia is a rare autoimmune platelet disorder in the patients with malignancies. Its clinical symptoms are thrombocytopenia, petechial purpura, and severe cutaneous hemorrhagic syndrome with multiple ecchymoses. The presence of fever, diffuse bone aches, asthenia, hepatosplenomegaly and multiple adenopathies are common in patients with this acquired thrombasthenia. Severe hemorrhagic episodes, with

a thrombasthenia-like profile, require transfusions with packed red cells, platelets, and fresh-frozen plasma. In the report of Andre et al. anti-GPIIb/IIIa complex antibodies were detected by direct MAIPA assay [75]. The antibody blocking active sites of GPIIb/IIIa was bound at the surface of platelets, and was potentially responsible for a fibrinogen-binding inhibition. The coagulation tests in this autoimmune disorder showed normal prothrombin time, activated cephaline time, and fibrinogen level, normal rate of soluble complexes and D-dimers, and nondetectable fibrin degradation products. A Simplate bleeding time is prolonged more than 20 minutes [75]. Like in the majority of malignant diseases, the treatment of underlying cancer should resolve immunological coagulation disorder. The methylprednisolone pulse therapy followed by maintenance dosing with intravenous prednisolone, gamma-globulin, and vincristine were described as effective in some TTP [73]. A severe immune thrombocytopenia with antiplatelet antibodies resistant to steroid and high-dose immunoglobulin therapy may be efficiently treated by splenectomy [72].

4.2 Autoimmune haemolytic anaemia

Autoimmune haemolytic anaemia (AIHA) is the most frequent form of autoimmune complication in B-CLL patients (66 %) [31, 76], splenic marginal zone cell lymphoma (SMZCL) [36], thymoma [73], or other lymphoproliferative disorders. The immune reaction was described to be triggered by oxaliplatin, when a relatively high cumulative dose of this agent has been administered [68].

Clinical presentation of disorder is characterised by the symptoms of haemolysis: hypotension, oliguria, hematemesis and darkly coloured urine, which corresponds to the presence of haemoglobinuria. As for idiopathic AIHA, the great majority of B-CLL-associated AIHA (89%) cases were due to warm autoantibodies (IgG+ direct antiglobulin test). Only 11 % were cold hemagglutinin diseases due to IgM polyclonal or monoclonal autoantibodies [31].

Laboratory tests should include standard haematologic tests, haemoglobin, white blood cell count, platelet count, the haptoglobin level, serum creatinine level and lactate dehydrogenase. The warm autoantibodies can be detected by direct antiglobulin test (DAT) for IgG and C3d/C3b, and cold hemagglutinin confirming IgM antibodies that demonstrate red cell agglutination at 3 degrees C by anti-C'+ direct antiglobulin test [68].

In the majority of patients a treatment of malignant disease should resolve immunological coagulation disorder. If haematologic event was a consequence of cytostatic therapy, drugs applied should be promptly discontinued [68]. Antibody production can sometimes be suppressed by chemotherapy. Recently, the use of anti-CD20 (rituximab) has found some success as the use

of fludarabine. In the case of IgM cold agglutinins, antibody production is not suppressed by prednisone and it should be used only when the simultaneous presence of IgG antibodies is found. Plasmapheresis can be used to remove antibody in some patients [77]. A special attention in any syndrome of cold-reacting antibodies is in keeping the patient warm [77].

Patients with progressive chronic lymphocytic leukaemia and life-threatening anaemia related to immune haemolysis can be treated with alemtuzumab, a humanised anti-CD52 monoclonal antibody [78]. The use of this monoclonal antibody was described in the treatment of severe immune complications of CLL unresponsive to corticosteroids, cytotoxic drugs, splenectomy, and immunoglobulins [76]. Monoclonal antibodies used in the AML therapy are described to lead to the bleeding complications. Gastrointestinal bleeding, epistaxis, CNS haemorrhage, and ocular bleeding were adverse events observed during combination therapy with anti-CD33 monoclonal antibody, gemtuzumab ozogamicin with cytarabine as continuous perfusion in elderly patients [79]. The bleeding or prothrombotic risk of the underlying haematologic disorder should be also considered during this immunotherapy. If haematologic toxicity or bleeding arises, escalation therapy with dose delays and reduction is recommended.

4.3. Haemolytic uremic syndrome vs. thrombotic thrombocytopenic purpura

Haemolytic uremic syndrome (HUS) is thrombotic microangiopathy characterized by microangiopathic haemolytic anaemia, thrombocytopenia and renal failure. It was described as a consequence of anticancer treatment, using gemcitabine [81], vincristine or L-asparaginase, and commonly in the childhood with *Escherichia coli* infection [82, 83]. Prodromes of typical HUS are bloody diarrhoea and fever, whereas atypical HUS presents with renal failure without diarrhoea. Drug reactions in cancer patients usually manifest as atypical form of HUS [82].

A diagnosis should be confirmed by renal biopsy and laboratory signs of haemolysis, reduced serum haptoglobin, elevated lactate dehydrogenase, negative direct Coombs test, and by absence of antiplatelet and anti-HLA antibodies. ADAMTS13 protease activity is normal (higher than 50%) or only slightly decreased. A histopathological investigation shows fibrin thrombi in small arteries in kidneys and in multiple organs, such as brain, heart, lungs, jejunum, liver, pancreas, adrenal glands and pituitary gland [69, 81]. The treatment of drug related HUS includes the discontinuation of the offending agent, supportive care, high dose corticosteroids, haemodialysis, fresh frozen plasma infusion (FFP) and immunosuppressive therapies [69, 81]. Some investigators prefer plasmapheresis to FFP infusions, although there's no clinical study favouring any method for the treatment of this rare disease [84].

Thrombotic thrombocytopenic purpura (TTP) is a coagulation disorder associated with classical symptoms: microangiopathic haemolytic anaemia, thrombocytopenia, neurological abnormalities, renal failure and fever [61]. In the absence of any apparent alternative cause, the combination of thrombocytopenia and haemolytic anaemia is enough to suggest the presence of TTP or HUS. If renal failure dominates the disorder is considered to be HUS [80].

Several studies reported that a severe deficiency of ADAMTS13 distinguishes syndromes described as TTP from the related syndromes described as HUS. ADAMTS13 cleaves a single Tyr-Met bond in domain A2 of the VWF subunit. If ADAMTS13 protease activity is insufficient (< 5%) due to acquired autoantibodies, platelet-rich microvascular thrombosis proceeds unchecked and TTP ensues [61] (see Fig. 3).

The clinical practice is inconsistent regarding the use of glucocorticoides and other immunosuppressive agents, partly due to the uncertain initial diagnosis in many patients. However, even among patients with acquired idiopathic TTP and severe ADAMTS13 deficiency, some respond promptly to short courses of plasma exchange, without additional glucocorticoid treatment. Patients with high-titre inhibitors may require glucocorticoids and more intensive immunosuppressive treatment with agents such as rituximab or cyclophosphamide [61].

4.4 Disseminated intravascular coagulation

Acute disseminated intravascular coagulation (DIC) is a clotting disorder characterized with both bleeding and intravascular thrombus formation. In cancer patients it is clinical manifestation associated with other noxious conditions i.e. irradiation, anticancer chemotherapeutic protocols, viral infections or surgical procedures. DIC is common with haematogenous malignancies like ALL [8], Burkitt's lymphoma [85], prostate cancer, and advanced or metastatic solid tumours like gastric cancer [11]. It also may appear in the decompensated phase of untreated immunologic events in malignancies as described in AIHA [68].

Patients present with venous or arterial thromboses, thrombocytopenia, and Coombs' positive haemolytic anaemia, commonly with raised anticardiolipin antibodies or positive lupus anticoagulant test. A decrease in fibrinogen levels and platelet counts, and increase of D-dimer, fibrinogen degradation products and soluble fibrin monomer supports the diagnosis [86]. Diagnosis should always be confirmed by finding abnormalities in at least 3 of those laboratory values.

Laboratory diagnostics: prolonged activated partial thromboplastin time, increased FDP, and decreased antithrombin-III. Platelet count in acute phase may not be changed. In chronic phase it is usually decreased [8]. The most

common observed signs of coagulation activation in the prostate cancer are TAT-complexes, D-dimer [87, 88], fibrinogen, and F1+2 fragments over normal range. After tumour removal or after complete remission (CR) of ALL was achieved, coagulation disorder will resolve. Transmission electron microscopy of the leukaemic blasts in patients with in a Burkitt's lymphoma/leukaemia showed crystalline cytoplasmic inclusions which may have a role in precipitating the disseminated intravascular coagulation, and immunological studies should revealed a B-cell phenotype with membrane bound IgM lambda [85].

There are no recommendations on plasma or platelet replacement in DIC. Current strategies do not support replacement therapy in DIC, but the treatment of primary disease. After successful treatment of malignant disease, DIC usually resolves. The platelets in DIC are always transfused if platelet count is lower than 50×10^9 [89].

Concluding remarks

Due to the growing occurrence of cancer patients and various chemotherapeutic protocols applied, immunological disorders should be considered as underlying to coagulation disturbances, since the treatment of these entities significantly differs from nonimmunological ones.

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