Pathophysiology of Trousseau's syndrome

C. Dicke; F. Langer

II. Medizinische Klinik und Poliklinik, Hubertus Wald Tumorzentrum – Universitäres Cancer Center Hamburg (UCCH), Universitätsklinikum Hamburg-Eppendorf, Germany

Keywords
Cancer, thrombosis, tissue factor, factor VII, microparticles

Summary
Clinically relevant clotting abnormalities in cancer patients are referred to as Trousseau's syndrome. While thrombotic complications such as venous thromboembolism are most frequent in every day's practice, cancer patients may also experience severe bleeding symptoms due to complex systemic coagulopathies, including disseminated intravascular coagulation, haemolytic thrombotic microangiopathy, and hyperfibrinolysis. The pathophysiology of Trousseau's syndrome involves all aspects of Virchow's triad, but previous basic research has mainly focused on the cellular and molecular mechanisms underlying blood hypercoagulability in solid cancers and haematological malignancies. In this regard, over-expression of tissue factor (TF), the principal initiator of the extrinsic coagulation pathway, by primary tumour cells and increased shedding of TF-bearing plasma microparticles are critical to both thrombus formation and cancer progression. However, novel findings on intrinsic contact activation in vivo, such as the release of polyphosphates or DNA by activated platelets and neutrophils, respectively, have pointed to additional pathways in the complex pathophysiology of Trousseau's syndrome.

Schlüsselwörter
Tumor, Thrombose, Tissue-Faktor, Faktor VII, Mikropartikel

Zusammenfassung
Klinisch relevante Gerinnungsstörungen bei Tumorpatienten werden unter dem Begriff Trousseau-Syndrom zusammengefasst. Während im Alltag thrombotische Komplikationen wie die venöse Thromboembolie im Vordergrund stehen, können als Folge komplexer Koagulopathien (z.B. DIC, hämolytische thrombotische Mikroangiopathie oder Hyperfibrinolyse) auch schwerste Blutungen auftreten. Die Pathophysiologie des Trousseau-Syndroms betrifft zwar sämtliche Aspekte der Virchow-Trias; die Grundlagenforschung hat sich aber vor allem mit der Hyperkoagulabilität des Blutes bei soliden und hämatologischen Malignomen beschäftigt. Diesbezüglich sind die Expression von Tissue-Faktor (TF), dem Initiator der extrinsischen Gerinnungskaskade, durch die Tumorzellen und die Freisetzung von TF-positiven Mikropartikeln sowohl für die Thrombusentstehung als auch für die Tumorprogression von zentraler Bedeutung. Neue Erkenntnisse über die molekularen Grundlagen der Kontaktaktivierung in vivo (z. B. Freisetzung von Polyphosphaten oder DNA aus aktivierten Plättchen und neutrophilen Granulozyten) deuten auf weitere Mechanismen in der komplexen Pathophysiologie des Trousseau-Syndroms hin.

Correspondence to:
Priv.-Doz. Dr. med. Florian Langer
II. Medizinische Klinik und Poliklinik, Hubertus Wald Tumorzentrum – Universitäres Cancer Center Hamburg (UCCH), Universitätsklinikum Eppendorf
Martinistr. 52, 20246 Hamburg, Germany
Tel. +49/(0)40/741 05-24 53, -06 64; Fax -51 93
E-mail: langer@uke.de

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Historical perspective

In 1865, the French physician Armand Trousseau described the association between superficial migratory thrombophlebitis and occult visceral malignancy (1). The original terms Trousseau's sign of malignancy or Trousseau's syndrome thus refer to the occurrence of an inflamed and thrombosed vein ahead of, or concomitant with, the diagnosis of cancer and highlight the potentially profound effects that hidden cancers may exert on the haemostatic system.

In 1977, Sack and colleagues (2) described their findings on 182 patients with malignancy-associated chronic disseminated intravascular coagulation (DIC). While at least a single episode of superficial thrombophlebitis was present in 113 patients (62%), the authors identified additional clinical features such as haemorrhage (41%), arterial emboli (25%), and non-bacterial thrombotic endocarditis (23%). Consistent with a state of systemic coagulation activation, various laboratory derangements were noted, including

- microangiopathic haemolytic anaemia,
- thrombocytopenia, and
- fibrinogen consumption (2).

Based on these observations, the term Trousseau's syndrome was extended to a more complex paraneoplastic syndrome with systemic coagulation activation, formation of platelet-rich microthrombi, and verrucous endocarditis.

In today's practice, however, virtually all clinically relevant clotting abnormalities in cancer patients are referred to as Trousseau's syndrome (3), including thromboembolic events in bedridden patients with end-stage solid malignancies. Because in these patients, the pathophysiology of Trousseau's syndrome is clearly different

from that in patients with occult or early-stage cancers or in those with haematological malignancies, it is not only important to acknowledge the term's evolution over time, but also to address specific aspects of Trousseau's syndrome in specific cancer subpopulations.

Clinical manifestations of Trousseau's syndrome

While the most common clinical manifestation of Trousseau's syndrome is venous thromboembolism (VTE), a composite of deep vein thrombosis (DVT) and pulmonary embolism (PE), cancer patients may also present with thrombosis in more uncommon sites, including veins of the upper extremities and those of the intracranial or visceral circulations. In addition and as already mentioned, cancer patients may experience complex systemic coagulopathies such as

- decompensated DIC,
- hyperfibrinolysis,
- haemolytic thrombotic microangiopathy, or
- non-specific proteolysis.

In general, arterial thrombosis is much less common than VTE in cancer patients, but such complications may occur without evident arteriosclerosis and in the complete absence of typical cardiovascular risk factors. A characteristic feature of malignancy is the consecutive or even concomitant occurrence of both thrombotic and haemorrhagic complications in a single patient. This coexistence is referred to as a thrombohaemorrhagic syndrome and poses a particular challenge to haematologists and oncologists.

Virchow's triad

The pathophysiology of Trousseau's syndrome is highly complex and, in most cases, involves all aspects of Virchow's triad (4). These are

- stasis or turbulent blood flow,
- alterations of the vessel wall, and
- blood hypercoagulability (▶Fig. 1).

Venous stasis is typical in immobilized patients with advanced malignancies and in those experiencing acute (e.g. infectious) complications during the course of their treatment as well as in surgical cancer patients. Blood flow may also be disturbed or completely interrupted by compression or direct invasion of the vessel wall by the tumour or by indwelling central venous catheters (CVCs) inserted for cancer treatment and/or patient nutrition.

In addition to CVC insertion, alterations of the vessel wall may further occur through tumour-derived inflammatory cytokines such as TNF-α or IL-1β and the direct action of cytotoxic drugs resulting in endothelial perturbation. For instance, cisplatin chemotherapy has been shown to induce apoptosis in cultured endothelial cells in vitro, including the shedding of procoagulant membrane-derived microvesicles (5), and to increase the risk for both venous and arterial thrombosis in vivo (6–8).

Of particular scientific and clinical interest, however, are the cellular and molecular mechanisms that underlie blood hypercoagulability. Malignancy is characterized by the destructive invasion of the vascularized organ tissue by cancer cells and the formation of leaky new blood vessels, so-called tumour angiogenesis. For these reasons, solid malignancies are typically described as wounds that do not heal. Furthermore, the procoagulant phenotype of cancer cells is likely to have a profound effect on the haemostatic system as a result from plasma leakage into the interstitial space of the tumour microenvironment, the secretion of inflammatory cytokines, chemokines, and carcinoma mucins, and the direct entering of cancer cells into blood or lymphatic vessels (3). In this regard, it has become clear that coagulation activation, inflammation, and tumour biology form a triangular network with reciprocal interactions between each of these components (9).

Tumour entities and VTE

From the literature it is difficult to conclude which tumour types are among the most thrombogenic. This uncertainty is probably caused by multiple factors.

1. In epidemiological studies on the incidence of VTE in cancer, it is almost impossible to dissect pathophysiological factors directly related to the cancer from those related to the patient or the cancer treatment.
2. The clinical stage of cancer, which grossly reflects tumour burden, is an important determinant of the patient's thrombotic risk, and many epidemiologic studies do not differentiate between patients with localized and those with locally advanced or metastatic tu-

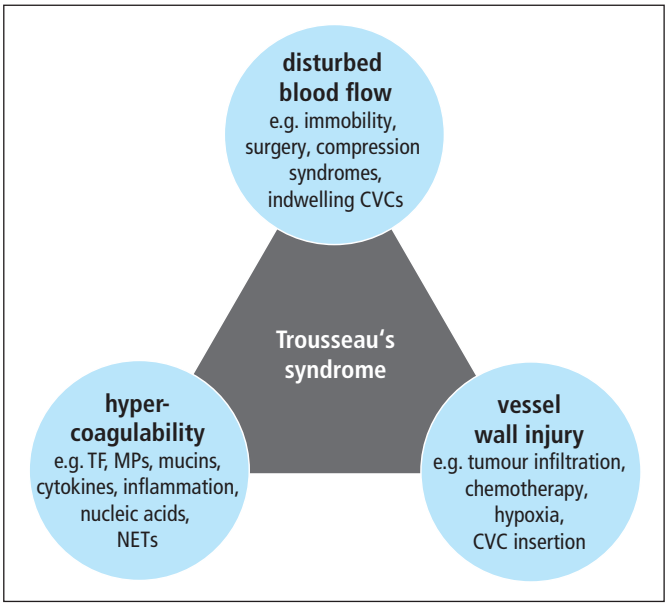

Fig. 1 Role of Virchow's triad in the pathophysiology of Trousseau's syndrome (CVCs: central venous catheters; MPs: microparticles; NETs: neutrophil extracellular traps; TF: tissue factor)

mours. For example, patients with localized prostate cancer are likely to have a rather low risk of VTE outside the surgical setting, while those with metastatic, hormone-refractory disease are at substantially higher risk (10) and may even present with complex systemic coagulopathies such as DIC (11, 12).

3. Epidemiologic studies may differ with regard to the inclusion of venous vs. arterial thromboembolic events and those events detected by clinical symptoms, routine imaging studies, or autopsy.
4. Cancer treatment has evolved over time and (potential) thrombogenic side effects of modern anti-angiogenic (e.g. bevacizumab, a humanized monoclonal VEGF antibody) or immunomodulatory drugs (IMiDs) such as thalidomide or lenalidomide have only recently become apparent (13).

Finally, even cancers of single organ systems are highly heterogeneous, not only from a histological or cytological, but also from a molecular standpoint. For example, lung cancer has formerly been grouped into small-cell and non-small-cell lung cancer (NSCLC), with the latter comprising the main histological subentities adenocarcinoma, squamous-cell carcinoma, and large-cell carcinoma. However, modern genetic and molecular analyses have identified further NSCLC subentities, particularly within the largest subgroup of adenocarcinomas, as well as specific molecular targets with therapeutic implications, including mutations in the gene for the epidermal growth factor receptor (EGFR) and translocations involving the gene for the anaplastic lymphoma kinase (ALK) (14). These novel findings could also be important for the pathophysiology of Trousseau's syndrome, because previous experimental and preclinical studies have delineated specific signalling pathways, by which activating or inactivating mutations in oncogenes (e.g. K-ras, EGFR, PML/RAR-alpha, MET) and tumour suppressor genes (e.g. p53, PTEN), respectively, directly affect the procoagulant phenotype of cancer cells (15).

Potential mechanisms of VTE in solid malignancies

Tumour TF expression

Despite the mentioned uncertainties and potential clinical confounders, some solid tumour entities appear to be associated with a particularly high risk of thrombosis. Among these are lung, pancreatic and stomach cancer, gynaecological cancers (in particular, ovarian cancer), and primary malignant brain tumours (i.e. high-grade glioma or glioblastoma multiforme, GBM) (16–19). In this regard, it is important to note that tissue factor (TF), the principal initiator of the extrinsic coagulation pathway, is over-expressed by each of these tumour entities (18–23). TF expression by these cancers appears to be correlated with the tumour grade (with poorly or undifferentiated cancers showing stronger TF expression) and the production of angiogenic growth factors such as VEGF (9). Furthermore, TF expression by primary tumours has been associated with the occurrence of VTE at least in some of the above-mentioned patient subgroups, providing circumstantial clinical evidence that cancer-derived TF indeed promotes thrombosis. Adenocarcinomas, particularly those of gastric or pancreatic origin, also secrete carcinoma mucins that have binding sites for selectins expressed on activated platelets and endothelial cells (P-selectin) or leukocytes (L-selectin). By promoting adhesive interactions between tumour cells, platelets, endothelial cells, and leukocytes within the vasculature, carcinoma mucins have not only been implicated in tumour progression, but also in the pathophysiology of Trousseau's syndrome (3, 24, 25). Additional molecular mechanisms, by which cancer cells may promote thrombosis, include over-expression of cyclooxygenase-2 (COX-2) and plasminogen activator inhibitor-1 (PAI-1) (15).

TF-bearing microparticles

TF is not only over-expressed by primary tumours, but also released into the bloodstream in association with sub-cellular membrane vesicles, so-called plasma microparticles (MPs), or as a soluble molecule (9). MPs are released from non-transformed cells following activation or induction of apoptosis, whereas the constitutive shedding of MPs is a characteristic feature of most cancer cells. MPs are typically enriched in procoagulant negatively charged phospholipids (i.e. phosphatidylserine) and may carry TF on their surface. Because cancer cell-derived MPs can easily enter the bloodstream through the leaky vessels of the tumour microenvironment, they have been the focus of tremendous basic and clinical research activity aiming at identifying the key players in cancer-associated thrombosis. Several studies have indicated that TF-bearing plasma MPs are indeed elevated in cancer patients with VTE or DIC (12,26), but it is currently not conclusively resolved if they are directly involved in the pathogenesis of Trousseau's syndrome or if they merely represent the systemic hypercoagulable and inflammatory state present in most patients with (advanced) solid malignancies. For instance, in an exploratory study involving 12 patients with NSCLC and no clinical evidence for acute bacterial infections, we evaluated MP-associated TF-specific procoagulant activity (MP TF PCA) before the initiation of anti-cancer therapy (▶Fig. 2). For this purpose, MPs were isolated from cryopreserved platelet-poor plasma by double high-speed centrifugation (2× 60 min at 16100×g) and analyzed for TF PCA by single-stage clotting assay in the presence or absence of inhibitory TF monoclonal antibody, essentially as described (12). Clotting times were calibrated against lipidated recombinant human TF and expressed as arbitrary units per ml of plasma. All patients provided written informed consent.

Compared to 12 age- and sex-matched controls, the median level of MP TF PCA was significantly increased in NSCLC patients (199 units/ml vs. 100 units/ml; $p < 0.05$ by Mann-Whitney U test) (▶Fig. 2). Importantly, the mean (± standard deviation) plasma level of C-reactive protein (CRP), an acute-phase reactant, was also increased to 45 ± 43 mg/l (normal, <5 mg/l) in NSCLC patients, indicating a systemic inflammatory response of the host to the tumour. Although patient numbers were too small to correlate MP TF

PCA and CRP levels with the clinical tumour stage (i.e. I/II vs. III/IV), these findings are consistent with the aforementioned triangular network formed by bidirectional interrelations between malignancy, inflammation, and coagulation.

With regard to the role of TF-bearing MPs in the pathophysiology of cancer-associated VTE, both clinical (26–29) and experimental animal studies (30, 31) have yielded somewhat conflicting results, with some (e.g. ferric chloride-induced injury of the mesenteric microvasculature or the saphenous vein), but not all of the murine models (i.e. ligation of the inferior vena cava) delineating a thrombogenic role of circulating TF-positive MPs in tumour-bearing mice. A comprehensive overview about clinical studies evaluating the association between TF-positive MPs and VTE in cancer, including those with positive and those with negative findings, has recently been provided by Geddings and Mackman (26).

Some of the discrepancies regarding the role of TF in cancer-associated thrombosis may be explained by the following factors:

- lack of standardization in assays to measure TF (e.g. antigenic vs. functional),
- differential contribution of tumour- vs. host-derived TF, and
- variations in the different pools of tumour-derived TF (i.e. TF expressed by primary cancer cells vs. TF circulating in association with plasma MPs vs. soluble TF).

Nevertheless, there is now compelling evidence in the literature that full-length TF is not only a key player in cancer-associated systemic coagulation activation, but also an important mediator of various cellular functions relevant to both inflammation and tumour progression (9). In contrast, the role of soluble TF in this context is currently unclear and still being investigated. The pool of circulating soluble TF may comprise alternatively spliced TF (asTF) and, possibly, a proteolytically degraded form of TF spanning (parts of) the extracellular domain (31, 32). Initial studies have suggested a role of asTF in thrombosis (33), but recent research has mainly focused on molecular mechanisms by which asTF promotes primary tumour growth, angiogenesis, and metastasis (34, 35).

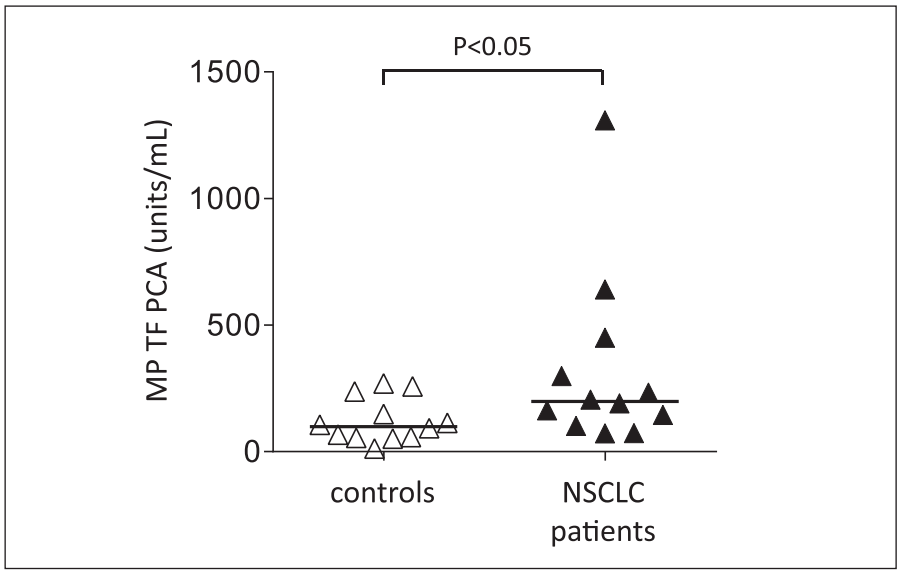

Fig. 2 Microparticle-associated TF procoagulant activity in NSCLC patients: Plasma microparticles (MPs) were isolated from 12 patients with non-small-cell lung cancer (NSCLC) before the initiation of anti-cancer therapy and analyzed for tissue factor-specific procoagulant activity (TF PCA) by single-stage clotting assay, essentially as described (12). Clinical tumour stages were localized (i.e. stage I/II) and advanced (i.e. stage III/IV) in 4 and 8 patients, respectively. The mean (± standard deviation) plasma level of C-reactive protein in NSCLC patients was 45 ± 43 mg/l (normal, <5 mg/l), indicating a systemic inflammatory host response to the tumour. The p value is according to Mann-Whitney U test.

VTE in high-grade glioma: a mystery?

The high incidence of VTE of 15–20% in GBM patients has long been, and probably still is, a mystery from a pathophysiological standpoint. GBM is characterized by strong TF expression, aggressive local growth with diffuse infiltration of the surrounding brain tissue by the cancer cells, and an overall poor clinical outcome, but does not normally form metastases at distant sites. Nevertheless, markers of systemic coagulation activation are elevated in GBM patients (36, 37), and increased levels of TF-positive plasma MPs, albeit likely not of glial origin, have been correlated with the occurrence of VTE in GBM patients (38, 39). Consistently, Thaler et al. (18) have not found an obvious association between in-situ TF expression by primary GBM cells and VTE in a subgroup analysis of their prospective Cancer and Thrombosis Study (CATS). A most recent study, however, may shed some light on these seemingly discrepant and unexpected findings. In this study, circulating tumour cells (CTCs) were found in the peripheral blood from 29 of 141 (20.6%) patients with GBM (40), an observation that could at least partially explain the development of extracranial metastases in organ transplant recipients from GBM donors (41–43). It is therefore tempting to speculate that TF-positive CTCs, and not circulating MPs, are a critical determinant of the thrombotic risk in GBM patients. Future studies specifically addressing the role of CTCs in the pathophysiology of Trousseau's syndrome are thus warranted.

Mechanisms of the thrombohaemorrhagic syndrome

AML/APL

CTCs are also a characteristic feature of haematological malignancies such as acute myelogenous leukaemia (AML), in which the malignant myeloblasts circulate in direct contact with the plasma compartment. It is thus very likely that the procoagulant (or fibrinolytic) phenotype of transformed

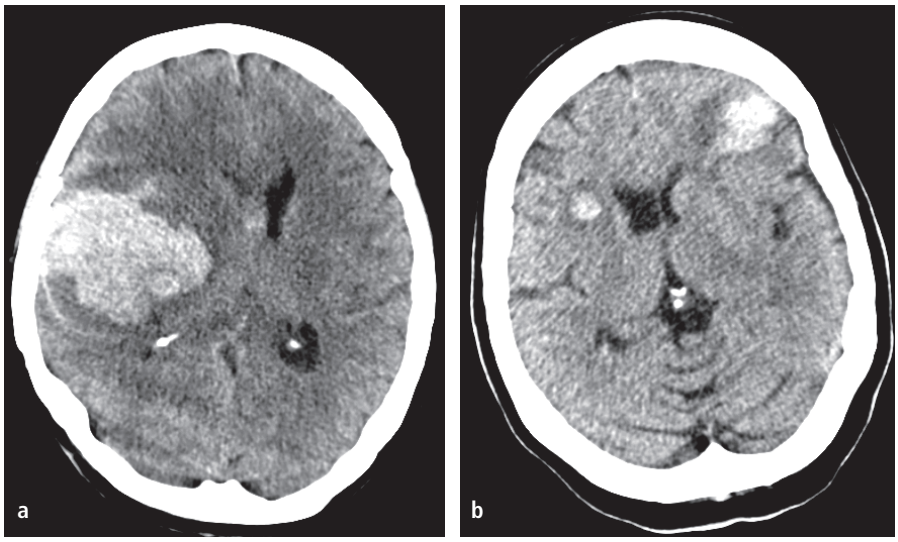

Fig. 3 Intracranial bleeding in two patients with AML and systemic coagulopathy: In both patients, haemostatic complications occurred before the initiation of effective anti-leukaemic therapy.
a) Fatal right-sided intracerebral haemorrhage (ICH) in a woman (age 61 years; peripheral blasts 97 × 10^3/µl) presenting with acute promyelocytic leukaemia and decompensated DIC.
b) Bilateral non-fatal ICH in a woman (age 74 years; peripheral blasts 287 × 10^3/µl) presenting with AML M1 according to the French-American-British classification. This patient also had decompensated DIC and eventually died from massive pulmonary embolism (thrombohaemorrhagic syndrome).

myeloid cells will have a profound effect on the haemostatic system in AML patients (►Fig. 3). In fact, over-expression of cellular TF has been implicated in the pathogenesis of AML-associated DIC (44, 45). In acute promyelocytic leukaemia (APL), constitutive TF expression can be regarded as a direct consequence of the quasi pathognomonic reciprocal translocation t(15;17)(q22;q21) involving the promyelocytic leukaemia (PML) gene located on chromosome 15 and the retinoic acid receptor-alpha (RAR-alpha) gene located on chromosome 17 (15). The resulting fusion protein PML/RAR-alpha can be targeted by all-trans retinoic acid or arsenic trioxide, which results in both differentiation of APL blasts into mature granulocytes and down-regulation of TF expression with subsequent resolution of the systemic coagulopathy (46, 47).

Aberrant TF expression has also been documented in other AML subtypes, mostly in those of (myelo)monocytic origin (44, 45), and appears to be required for the evolution of decompensated DIC in patients with newly diagnosed AML (48). Recently, circulating TF-bearing plasma MPs have also been implicated in the pathogenesis of AML-associated DIC (12, 49, 50), but their true relevance in relation to the potentially high numbers of circulating blasts needs to be determined in future studies. The systemic coagulopathy of AML, and particularly that of APL, is further complicated by the expression and secretion of various fibrinolytic factors, including plasminogen activators (uPA and tPA), urokinase receptor (uPAR), annexin A2, and S100A10 (45, 51). Excessive fibrinolysis in AML should thus not be solely regarded as a secondary response to TF-driven coagulation, but rather as an independent mechanism actively promoted by AML blasts.

Myeloma and lymphoma

The pathophysiology of thrombosis in haematological malignancies such as myeloma and Hodgkin or non-Hodgkin lymphoma is less clear, because a distinct intrinsic hypercoagulability has not yet been characterized in these lymphoproliferative disorders. Unstimulated lymphocytes and malignant plasma cells do not appear to overexpress TF (52, 53). It is thus likely that additional factors such as compression syndromes, CVCs, infectious complications, chemo-/radiotherapy, and treatment with IMiDs determine the risk of VTE in myeloma and lymphoma patients (54).

Myeloproliferative neoplasms

Essential thrombocythaemia (ET) and polycythaemia vera (PCV) are clonal myeloproliferative neoplasms (MPNs) characterized by an increased risk for both venous and arterial thrombosis, particularly in atypical sites, as well as for microcirculatory disturbances such as erythromelalgia, migraine, and tinnitus (55). The activating mutation, V617F, in the cytoplasmic tyrosine kinase, Janus kinase 2 (JAK2), which is required for efficient intracellular signal transduction by various haematopoietic growth factors, including erythropoietin, thrombopoietin, and granulocyte colony-stimulating factor (G-CSF), can be found in >95% and 50–60% of PCV and ET patients, respectively. The prothrombotic state in ET and PCV patients probably results from a complex interaction of the mutated haematopoietic stem cell clone with the normal bone marrow microenvironment, evoking an inflammatory host response that feeds back on the prothrombotic profile of both clone-derived circulating blood cells and normal vascular cells such as platelets, leukocytes, and endothelial cells (55). Consequently, increased platelet activation and platelet-leukocyte-conjugate formation have been described in ET patients (56). In this regard, platelets from JAK2-V617F-postive patients showed higher baseline P-selectin expression and were more responsive to exogenous stimuli than platelets from JAK2-V617F-negative patients (56), a finding most likely explained by a higher percentage of immature platelets in carriers of the JAK2-V617F mutation (57). Consistently, Rumi and colleagues (58) have most recently shown that ET patients with the JAK2-V617F mutation, as compared to patients with mutations in the calreticulin (CALR) gene, have a two-fold higher risk of thrombosis, which is similar to that of PCV patients. Increased TF production, probably resulting from the systemic inflammatory host response, and shedding of procoagulant MPs have also been implicated in the pathogen-

esis of ET-associated thrombosis and inflammation (59–61), while disturbed blood flow due to an elevated haematocrit is considered a major risk factor for vascular complications in PCV patients (62).

Role of the intrinsic contact pathway

Previous research has clearly focused on the role of the extrinsic, TF-driven pathway of coagulation in the pathophysiology of Trousseau's syndrome. Initial enthusiasm on a potential role of cancer procoagulant, a cysteine proteinase with direct factor X-activating activity, in cancer-associated thrombosis has somewhat subsided over the past decade. In contrast, the relevance of the intrinsic coagulation pathway for both venous and arterial thrombosis has recently gained tremendous attention. In vivo, contact activation of factor XII relevant to the pathogenesis of VTE may be achieved by negatively charged polymers such as polyphosphates or nucleic acids (i.e. RNA, DNA) liberated from bacteria, activated platelets and leukocytes, or necrotic cells (63). In particular, extracellular DNA released from specifically stimulated neutrophils in association with neutrophil extracellular traps (NETs) has most recently been implicated in the pathogenesis of VTE in general and Trousseau's syndrome in particular (64,65). NETs are composed of DNA fibres and protein components such as histones, myeloperoxidase, neutrophil elastase, and cathepsin G (66).

Animal models of venous thrombosis suggest that NETs released from recruited neutrophils at sites of activated vascular endothelium may promote thrombus formation by providing an additional scaffold for platelet and red blood cell adhesion, by mediating fibrin deposition, and by enhancing activation of platelets and endothelial cells (67). In addition, elevated levels of cell-free DNA, a plasma biomarker of NETs, have been found in various haemolytic thrombotic microangiopathies (TMAs), including cancer-associated TMA (68). Elevated leukocytes are a risk factor for VTE in ambulatory cancer patients (69), and circulating nucleosomes and neutrophil activation are associated with VTE occurrence in cancer and non-cancer patients (70), providing circumstantial clinical evidence that NETosis may indeed be involved in the pathophysiology of cancer-associated VTE. However, considering that patients with chronic myelogenous leukaemia (CML), an MPN characterized by the BCR/ABL fusion protein and exceedingly high numbers of circulating neutrophils, are not at particular risk for VTE when compared to ET and PCV patients, further research is needed to define the exact contexts and mechanisms of extracellular DNA trap formation and contact activation in Trousseau's syndrome.

Screening for occult malignancy in idiopathic VTE?

Considering that, in its original meaning, Trousseau's sign of malignancy precedes the diagnosis of cancer and that approximately 10% of apparently cancer-free patients with unprovoked VTE will be diagnosed with a malignant disease within the next 1–2 years, the question arises of whether patients with spontaneous (migratory) thrombophlebitis, DVT or PE should be screened for occult malignancy.

Several studies have addressed this issue. For example, Piccioli et al. (71) compared extensive tumour screening, including computed tomography (CT) scanning of the thorax, abdomen and pelvis, gastro- and colonoscopy, sputum cytology, mammography, and tumour markers (e.g. CEA and PSA), with no screening in 201 patients with idiopathic VTE. Initial detection of occult cancer was significantly more frequent in the extensive screening as compared to the control group (13% vs. 0%), whereas 1.0% and 9.8% of patients in the screening and control group, respectively, developed symptomatic cancer over the subsequent two years ($p < 0.01$). Although cancers in the screening group were detected at an earlier stage, there was no significant difference in cancer-related mortality between the screening (2.0%) and the control group (3.9%).

In a more recent study on 630 patients, van Doormaal et al. (72) added chest and abdominal CT scanning and mammography to a baseline screening program consisting of medical history, physical examination, basic laboratory tests, and chest X-ray. Initial detection of malignancy (3.5% vs. 2.4%) and cancer-related mortality over a median 2.5 years of follow-up (5.0% vs. 2.8%) were similar between the extensive and the limited screening group.

> Based on these and other observations, an evidence-based mini-review recommended that all patients with unprovoked VTE provide a thorough medical history and undergo physical examination, chest X-ray, and routine laboratory testing, including complete blood count, basic chemistries, lactate dehydrogenase, and liver enzymes (73).

In addition, age- and gender-specific cancer screening should be up-dated, and clinicians should maintain an overall low threshold of suspicion for occult malignancy in patients with unprovoked VTE. Additional diagnostic tests should be ordered based on any abnormal finding from the initial clinical and laboratory work-up. In this regard, simple and easily obtainable variables such as bilateral thrombosis, anaemia, and patient age of 60–75 years have been associated with an increased risk for hidden cancer in the prospective RIETE registry (74).

Future perspectives

As stated by Ajit Varki in his comprehensive review, there are multiple definitions and multiple mechanisms of Trousseau's syndrome (3). For this reason, there will not be any single molecule or pathway that can be held accountable for the plethora of paraneoplastic clotting abnormalities. Nevertheless, tremendous progress has been made in delineating (some of) the cellular and molecular events underlying cancer-associated micro- and macrovascular thrombosis, many of which already have therapeutic implications. In this regard, a

- standardization of techniques to measure TF,
- thorough understanding of how the extrinsic and the intrinsic coagulation

pathway cooperate to trigger intravascular thrombosis and
- an assessment of how CTCs contribute to the hypercoagulable state in general and to the pathophysiology of cancer-associated VTE and TMA in particular are needed.

In addition, specific biomarkers such as TF, carcinoma mucins, or cell-free DNA should be evaluated as complementary diagnostic tools in patients with unprovoked VTE to guide further work-up for hidden cancer or in patients presenting with presumed, but not yet proven malignancy. For instance, in a prospective pilot study involving 55 women with ovarian masses of unknown aetiology, all of whom underwent exploratory laparotomy, we found that combined analysis of preoperative CA125, plasma D-dimer, and MP-associated TF PCA improved the diagnostic accuracy for ovarian cancer (75).

Conflict of interest

The authors declare that they have no conflicts of interest relevant to the content of this article.

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