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Tissue factor-bearing microparticles and inflammation: a potential mechanism for the development of VTE in cancer

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Abstract

Cancer is associated with an increased risk of venous thromboembolism (VTE); the exact mechanisms for the induction of VTE remain to be fully elucidated, yet it is widely acknowledged that tissue factor-positive microparticles (TF-MPs) may play a significant role. However, TF-MPs have yet to be accepted as a bona fide biomarker for cancer-associated VTE, as the presence of elevated TF-MPs levels is not always accompanied by thrombosis; interestingly in certain cases, particularly in pancreatic cancer, VTE seems to be more likely in the context of acute inflammation. While several potential mechanisms for the development of VTE in cancer have been postulated, this review explores the homeostatic disruption of TF-MPs, as the main reservoir of blood-borne TF, in the context of cancer and inflammation, and considers the abrogated responses of the activated endothelium and mononuclear phagocyte system in mediating this disruption.

Keywords: cancer, inflammation, microparticles, thrombosis, tissue factor.

DVT formation *in vivo*

The exact mechanisms that induce clotting in venous thromboembolism (VTE) have yet to be fully elucidated. Although arterial thrombosis is shown to be provoked by endothelial disruption, a frequent model described for deep vein thrombosis (DVT) is venous stasis in the absence of endothelial injury (except in thrombosis associated with surgery) [1, 2]. The most common site for DVT initiation in humans is the valve pocket sinuses of the calf veins due to periods of blood stasis and low oxygen levels, and therefore a predisposition to become hypoxic [3]. The endothelium is activated by hypoxia and/or inflammatory stimuli, leading to the expression of adhesion receptors platelet selectin (P-selectin), endothelial selectin (E-selectin) and von Willebrand factor (vWF) on the endothelium that facilitate the binding of

circulating leukocytes (specifically neutrophils and monocytes), platelets and microparticles (MPs) [4-6]. In contrast, the normal endothelium prevents thrombosis by inhibiting the attachment of cells and proteins necessary for clotting, expressing anticoagulants (including tissue factor pathway inhibitor [TFPI], thrombomodulin (TM), endothelial protein C receptor, and heparin-like proteoglycans), and releasing platelet inhibitors (nitric oxide and prostacyclin) [7, 8]. However, following endothelial cell (EC) activation, TM is downregulated, while expression of tissue factor (TF), the primary initiator of the extrinsic coagulation cascade, is increased on the cell surface [9], shifting the haemostatic balance towards a hypercoagulable state. In addition, the endothelial-bound leukocytes become activated and express TF, initiating the activation of the coagulation cascade, and inducing thrombosis [10, 11].

This proposed sequence of events is supported by a recent novel study employing a mouse inferior vena cava (IVC) stenosis model for DVT initiation and propagation [11], identifying neutrophils as the predominant leukocytes recruited to the activated endothelium and present during the early stages of thrombosis. The study found that in mice expressing minimal levels of TF on myeloid leukocyte cells, the development of venous thrombosis was considerably reduced, implying that leukocyte-derived TF plays a key role in the initiation of thrombus formation in this model. Additionally, von Bruhl *et al*. confirm that platelets contribute to DVT propagation by binding to leukocytes and promoting secondary leukocyte recruitment at the thrombus site and, in agreement with others, suggested that neutrophils amplify DVT development by forming neutrophil extracellular DNA traps (NETs), which trigger Factor XII (FXII)-dependent coagulation via the contact pathway [11], and induce activation and aggregation of platelets via their constituent histones [12]. Indeed the specific depletion of neutrophils in a murine DVT model, and the degradation of NET scaffolds following treatment with DNase I or heparin, both resulted in a marked inhibition of thrombus formation [11, 12].

VTE in cancer

The prevalence of VTE in cancer is around four- to five-fold higher than in the general population [13]. This generally confers a poor prognosis and constitutes the second leading cause of death in cancer patients [14]. The pathogenesis of VTE in cancer patients is most likely a result of the interactions between cancer cells and host factors, which may be framed in the context of "Virchow's triad" [15] consisting of 3 elements: venous stasis, endothelial injury/abnormality and hypercoagulability, which can all simultaneously account for the prothrombotic state in the same cancer patient. Venous stasis has been suggested to contribute to venous thrombosis in cancer that may manifest from immobility during a post-operative period or debility in advanced malignancy, extrinsic compression of blood by the primary tumour, or tumour invasion. Furthermore, slow venous blood flow can lead to the improper clearance of activated clotting factors causing hyperviscosity and increasing incorporation of MPs into endothelial membranes resulting in the concentration of these factors at the endothelial surface. Accumulated clotting factors in stagnant blood could overwhelm local anticoagulant pathways, thus inducing a hypercoagulable state of malignancy that may trigger thrombosis. Endothelial injury may promote thrombosis in cancer patients as a result of mechanical trauma arising from tumour invasion or therapeutic interventions such as surgery or placement of venous catheters for chemotherapy administration, while endothelial dysfunction can also result from antiangiogenic stimuli and the 'unselective' effects of some chemotherapeutic agents that are toxic to ECs. All proposed mechanisms relating to the pathogenesis of VTE can be traced back to abnormalities in at least one of these triad components.

A multitude of clinical and biological risk factors for the development of VTE in cancer patients have been identified and have been extensively reviewed [16, 17]. In addition to general VTE risk factors, cancer-driven events found to specifically promote a hypercoagulable state

include the increased activation of procoagulant factors with concomitant inhibition of anticoagulant mechanisms, impaired fibrinolysis, production and secretion of procoagulant substances and proinflammatory cytokines, increased platelet aggregation and adhesive interactions among tumour cells, endothelium and blood cells [15]. Oncogene and tumour suppressor gene-mediated neoplastic transformation events (e.g. activation of Met, loss of PTEN, induction of K-ras, loss of p53) [17] can also promote haemostatic derangement, as can inherited prothrombotic variants (e.g. factor V Leiden and prothrombin G20210) or deficiencies in antithrombin, protein C or protein S [2, 16]. Anti-cancer treatments, such as chemotherapy, hormonal therapy, anti-angiogenic therapy, erythropoiesis stimulating agents, blood transfusions and central venous lines have also been found to contribute to an increased risk.

VTE risk is found to vary depending on the primary cancer site and stage of disease. While the highest rates are reported in pancreatic (5.3% to 26.0%) and brain (1.6% to 26.0%) cancers, breast (0.4% to 8.1%) and prostate (0.5% to 1.4%) cancers are found to have the lowest rates [16]. Rates among patients with advanced metastatic cancers are higher than in those with localised tumours, and histological grading also seems to be an important factor; analysis of data from the Vienna CATS study found the risk of developing VTE to be twice as high in patients with high-grade tumours (G3 and G4) compared to those with lower grade tumours (G1 and G2) [18].

A number of discriminatory parameters (e.g. tumour entity, BMI, haemoglobin level, leukocyte count) and putative biomarkers (e.g. D-dimer, prothrombin fragment 1+2, soluble P-selectin, TAT complexes, TF-bearing MPs) for VTE risk have been proposed, and several have been incorporated into risk assessment scores, designed to aid in identification of those cancer patients at high risk of thrombosis, with the aim of delivering more targeted thromboprophylaxis. However a recent evaluation of current risk scores in a multinational

prospective cohort study of patients with advanced solid cancers highlighted their poor discriminatory capacity, particularly when studying individual tumour types [19]. This emphasises the need to investigate further putative mechanisms, which can be used to develop more refined, and possibly even cancer-type specific models for risk prediction.

The differences observed between cancer types suggest that identified causes of VTE may in fact have varying levels of influence, dependent on the cancer entity, and that VTE may even be triggered by distinct cancer type-specific mechanisms. In a number of cancers, and especially in pancreatic cancer, one of the most highly characterised mechanisms is the increased activation of the TF coagulation pathway, via the upregulation of TF on the surface of tumour cells, and subsequent release of TF-bearing MPs (TF-MPs). In contrast, in malignant gliomas, another highly prothrombotic malignancy type, Riedl *et al*. [20] suggest that the coagulation activation observed may be less reliant on TF, and may instead be attributed at least in part to high intratumoural podoplanin expression, which induces platelet activation and aggregation, and was found to correlate with hypercoagulability. In prostate cancer, which has a comparatively much lower associated risk of VTE, the polyphosphate-factor XII pathway is postulated as a main driver of thrombosis [21].

The contribution of TF to cancer-associated thrombosis

TF expression can be induced in a variety of cell types, and recent evidence from *in vivo* studies suggests that not only arterial, but also venous thrombosis is initiated by intravascular TF, involving multicellular interactions at the site of ongoing thrombosis [11, 22]. Cancer-associated thrombosis is frequently attributed to high levels of TF expression, involving primarily the upregulation of TF on the surface of tumour cells and their migration into the vascular lumen during vascular invasion and metastasis, but also TF-positive inflammatory and

stromal cells within the tumour microenvironment, upregulated tumour vasculature TF expression by angiogenic or activated ECs, and the release of TF-MPs into the blood circulation [23-28].

Patients with pancreatic cancer are at a particularly high risk of developing VTE when compared to other cancer types [29]. Pancreatic cancer cells often exhibit markedly high levels of TF expression [30-32], which have been explicitly linked to procoagulant activity *in vitro* [33], while elevated TF expression in pancreatic cancer patients has been positively correlated with the rate of symptomatic VTE [32]. In addition, elevated levels of tumour-derived TF-MP can be detected in the circulation of mice bearing human pancreatic and lung cancer cell lines and tumours [34-36] and it is these procoagulant MPs, rather than the parental tumour cells, that are found within sites of ongoing thrombosis *in vivo* [34, 37, 38], and have been shown to accumulate at sites of injury in a P-selectin-dependent manner where they aggregate platelets and accelerate thrombus growth [34]. The critical role of TF is emphasized by a significantly reduced incidence of thrombus formation in mice perfused with low-TF Panc02-derived MP compared with unmodified Panc02-derived MP in a murine IVC stenosis-pancreatic cancer model [38].

A definitive link between TF-MPs and the development of clinical VTE has yet to be established and remains somewhat debated in view of conflicting evidence, as summarised in a recent review [39]. Differences in association could be due to varying levels of TF encryption and participation of TF in non-coagulant activities, such as signalling of the TF-factor VIIa (FVIIa) complex via protease-activated receptor 2 (PAR2) [28]. In pancreatic cancer, increased levels of both TF-MPs and MP-TF activity have been significantly associated with VTE [40-42] and, furthermore, radical pancreatectomy in pancreatic cancer patients results in a significant decrease in TF-MP levels [41], which is accompanied by a significant reduction in PCA [43]. The exact contribution of TF-MPs to the development of VTE may therefore be highly variable

between tumour types, and the association in pancreatic cancer may reflect the high level TF expression relative to other cancer types and/or the late stage of disease presentation [16]. Somewhat surprisingly however, while around 80% pancreatic cancer patients show increased TF levels, fewer than 30% of these patients are found to develop thrombosis [44], indicating that elevated TF-MP levels in isolation do not unequivocally trigger thrombosis, and raising the interesting question of why the clinically recorded thrombosis rate is so much lower. Intriguingly, the occurrence of a thromboembolic event in these patients is often accompanied by the presence of persistent inflammation.

Putative role of inflammation in promoting DVT

While TF-MP likely participate in thrombus propagation in pancreatic cancer, it is unlikely they represent the sole causative mechanism; indeed several other factors are proposed to promote a hypercoagulable state in this context [45]. It is therefore more likely that TF-MPs constitute an important contributory factor among other integral components within a multifactorial process.

Inflammation is almost invariably accompanied by alterations to the coagulation system, and therefore may have a further compounding effect in the context of malignancy, an already hypercoagulable state, which could thereby precipitate thrombosis. Furthermore, once activated, coagulation pathways can markedly affect inflammatory activities, establishing a cycle of sequential aggravation to further amplify the host response and potentially increase the risk of VTE.

Inflammation induces coagulation

The presence of inflammation in cancer is characterized by changes in cell numbers in peripheral blood and levels of inflammatory cytokines. The findings of a study by von Bruhl *et*

al. suggest that massive leukocyte accumulation precedes the development of DVT in response to perturbed venous blood flow [11]. Pabinger and Posch [46] analysed the Vienna CATS study dataset to show an increase of 1 x 10^9/L in WBC to be associated with a 7% increase in risk of VTE, while the Tromsø study interestingly noted that an elevated WBC count measured even prior to the development of cancer was also associated with a future risk of VTE [47], hinting that leukocytes are not simply a reflection of low-grade inflammation, but more likely have a potential causal role.

A key effect of inflammatory mediators is to induce the expression of TF on the surface of circulating monocytes, tissue macrophages and neutrophils, increasing the pool of TF potentially available to participate in coagulation. Upregulation of TF expression has been specifically demonstrated *in vitro* on human peripheral blood monocytes in response to proinflammatory stimuli [48-50], while TF production by neutrophils attached to an injured endothelium is recognized as an initiatory event in thrombus formation in DVT models [22]. Moreover, circulating mononuclear cells harvested from V2 carcinoma-bearing rabbits were found to generate significantly more PCA than those from control tumour-free animals [51].

On exposure to inflammatory cytokines, leukocytes are also more prone to microvesiculation [52] and hence thereafter capable of producing TF-MPs, which may be recruited to growing thrombi via P-selectin glycoprotein ligand 1 (PSGL1)-P-selectin interactions [53], and may serve to stabilize the thrombus by inducing fibrin formation [54]. Neutrophils can also recruit TF-MPs [55], while the NETs they expel have been shown *in vitro* to serve as a site of adherence for tumour-derived TF-MPs [38]. This could be an important process for localizing TF-MPs and concentrating additional TF into the vicinity of the developing clot.

There is also evidence to suggest that MPs are able to transfer their procoagulant potential to other cell types [54] and in doing so can exacerbate endothelial activation. While TF expression is seen to be induced in cultured ECs in response to inflammatory mediators such as TNFα and

LPS endotoxin [56, 57], *in vivo* however it seems more likely that the TF associated with ECs is derived from TF-MPs released by monocytes or tumour cells [58-60]. It has been demonstrated that TF-MPs can arise from lipid raft regions in monocyte cell membranes and fuse with activated platelets enabling transfer and subsequent expression of their membrane protein complement (including TF), which can serve to initiate coagulation [61]; it is possible these MPs may interact with ECs in a similar manner. Others have suggested that exposure of acquired TF on the endothelial surface may occur via an endocytic recycling process [62], through which surplus circulating TF-MPs from cancer patients can cause upregulation of endothelial-expressed TF through activation of the PAR2 pathway [63], enhancing the coagulopathic effect. Many of the inflammatory processes that accompany the thrombosis process in cancer have the propensity to activate this pathway. At particularly high levels of TF, such as those expressed by tumour cells and induced by high levels of proinflammatory factors, this PAR-2 driven mechanism may become saturated. This may result in accumulation of TF in ECs which compromises their ability to release TF in the form of endothelial TF-MPs, and instead serves to promote pro-apoptotic mechanisms within ECs via upregulation of Bax [64], leading to endothelial denudation [65] and exposure of the sub-endothelial tissues (i.e. a thrombogenic surface) to blood flow.

The normal resting endothelium is essentially anticoagulant. Expression of TF on the endothelium in response to both monocyte-derived MPs and inflammatory mediators is accompanied by concomitant translocation of phospholipids such as phosphatidylserine (PS) which may serve to enhance the binding of coagulation factors [66], while the induction of EC apoptosis is associated with MP generation, and downregulation of TFPI, TM and glycosaminoglycans such as heparan sulphate on the endothelial surface [67], with resultant impairment of activation of the protein C anticoagulant pathway and reduced antithrombin III activity, which may be partly attributed to the disrupted integrity of the endothelium. Activated ECs are also induced to express surface adhesion molecules which increase platelet

adhesion and attract monocytes and neutrophils, all of which may contribute further to coagulation initiation or amplification [66, 68]. At this point, the endothelial recycling process is overwhelmed and the innate anticoagulant mechanisms are no longer sufficient to counteract the procoagulant driving force of the TF. The physiological antithrombotic state is compromised, with the net effect that, during inflammation, the EC phenotype switches from thromboresistant to procoagulant [67].

Neutrophils in particular are found in large quantities in the plasma of cancer patients, and are recruited in the earliest stage of an inflammatory response [11]. Interestingly, a recent study found that tumour-induced neutrophils are more prone to NET formation that their normal counterparts in both leukaemia and solid tumour (mammary and lung carcinoma) experimental models, thereby promoting a cancer-associated hypercoagulable state [69]. These NETs have demonstrable roles in thrombosis, providing a scaffold closely intercalated with the fibrin matrix, enhancing thrombus formation and subsequent stabilisation, and facilitating the adhesion of platelets and erythrocytes [12]. Additionally they are seen to alter clot morphology, producing denser clots with reduced permeability and increased resistance to tissue plasminogen activator (tPA)-driven lysis [70], which will potentially persist for longer in the circulation, exacerbating the thrombotic effect. This prothrombotic fibrin clot phenotype has been observed in digestive tract cancers [71] and in multiple myeloma [72], while NETs themselves have been identified in a number of cancers, including pancreatic cancer [73], and pancreatic cancers cells have been shown to stimulate rapid release of NETs, promoting thrombus formation under venous flow conditions [74].

The role of NETs in cancer-associated thrombosis has recently been challenged by Nouboussie *et al*., who argue that it is the constituent products of NETs (i.e. cell-free DNA [cfDNA] and histones), and not intact NETs, that are able to activate coagulation [75]. However, as the authors admit, this observation was made only in an *in vitro* setting, and therefore does not

take into account the compounding effects of potential interactions with other relevant cells and effects of venous flow *in vivo*.

However, neutrophils do not represent the only source of cfDNA in plasma, and other forms of cfDNA may contribute towards the prothrombotic state. Elevated levels of cfDNA in cancer patients have additionally been characterised as either tumour-derived or released from injured host blood or vascular tissues via apoptotic and necrotic cell death [76], and are thought to provide an activation surface for the contact pathway of coagulation. The effect of chronic inflammation in the tumour microenvironment is proposed to be similar to that observed in response to chronic exercise protocols, characterised by a slow, constant release of DNA via apoptosis or necrosis [77]. In contrast, intense exercise results in a rapid and transient increase of cfDNA which is thought to be attributed, at least in part, to NETosis [78]. It is possible that the constant cfDNA release occurring in the chronically inflamed tumour microenvironment may be exacerbated by the addition of an acute inflammatory stress, evoking further spontaneous cfDNA release in the form of NETs. In the acute exercise setting in healthy individuals, this is effectively counterbalanced by a concomitant increase in serum DNase activity [78], but the absence or inadequacy of this mechanism in cancer, accompanied by the persistence of neutrophils in the circulation, will likely prolong the effect.

NETs also serve to locally concentrate neutrophil serine proteases, which are responsible for further promoting coagulation, again by directly counteracting endogenous anticoagulants [79]. Neutrophil elastase (NE), cathepsin G (CG) and proteinase-3 (PR3) have all demonstrated an ability to cleave and inactivate EC-bound TFPI [79, 80], while PR3 has additionally been found to induce expression of encrypted TF on the EC surface [80], thus shifting the balance even further in favour of thrombosis. Disruption of the TF:TFPI ratio is common in several inflammatory disease conditions, including sepsis, acute myocardial infarction and disseminated intravascular coagulation (DIC) [81-83].

Coagulation exacerbates inflammation

In complex with blood coagulation Factor VIIa (FVIIa), TF can also play an important role in intracellular non-haemostatic signalling pathways. This can lead to activation and induction of further inflammation, and can induce proangiogenic and immune modulating cytokines, chemokines and growth factors, promoting proliferation and invasiveness of cancer cells [28]. This effect is largely mediated by the ability of coagulation serine proteases to interact with PARs on the surface of monocytes and ECs [84]. Activation of multiple PARs by coagulation proteases is thought to contribute to inflammation in endotoxaemia and sepsis [85], and similarly may do the same in cancer.

As such, high levels of TF in the form of TF-MPs can contribute to the amplification of inflammation via induction of PAR1 and PAR2 on ECs, through generation of thrombin and Factor Xa (FXa) respectively [84, 86]. This is dependent on co-localization of PAR receptors with their activating proteases [87], facilitated by expression of adhesion molecules and enhanced leukocyte trafficking by the activated ECs. Activation of PAR1 and PAR2 in turn contributes to the induction of acute pro-inflammatory responses within the endothelial layer, induces expression of proinflammatory cytokines and chemokines [85], exposing further cell adhesion molecules [88] and eliciting production of ROS [89], and increasing susceptibility to MP endocytosis.

PAR2 is also susceptible to activation by neutrophil serine proteases. While NE, CG and PR3 have been shown to disarm PAR2 and target it for internalization, attenuating G_q-coupled PAR2 calcium signalling in response to trypsin [90], NE possesses the additional ability to evoke PAR2-mediated $G_i/G_{12/13}$-coupled activation of the p44/42 mitogen-activated protein kinase (MAPK) pathway and subsequent extracellular signal-related kinase (ERK) phosphorylation, independent of β-arrestin recruitment and receptor internalization [90]. In this respect, NE is able to demonstrate biased agonism, and it is possible that, under inflammatory conditions,

accompanied by increased neutrophil infiltration and associated high levels of neutrophil serine proteases, the balance of PAR2 signalling may be shifted towards NE- rather than trypsin-triggered MAPK signalling, targeting nuclear as opposed to non-nuclear ERK substrates [90].

The mononuclear-phagocyte system

Monocytes, along with macrophages and dendritic cells, form an integral part of the mononuclear phagocyte system (formerly known as the reticuloendothelial system) and form a significant presence in the tumour lymphoreticular infiltrate [51]. This is a filtration system for the removal of insoluble cellular debris and particulate matter, classically responsible for the clearance of platelets [91]. It now appears that both apoptotic bodies and MPs follow a similar fate [54, 92], cleared from the blood by macrophages in the liver and spleen. Indeed evidence suggests that MPs are continuously formed and rapidly cleared from the circulation *in vivo* [93-95], and it also appears that the system may be responsible for the clearance of activated clotting factors such as thrombin [96] and also of fibrinogen-fibrin complexes [91].

It follows that any TF in association with MP, and indeed any products of the coagulation process, under normal circumstances, are unlikely to remain in the circulation for long enough to accumulate to a level capable of promoting clot formation [63]. The presence of PS on the surface of TF-MPs, although enhancing their procoagulant potential [54], also labels them for phagocytic clearance [92]. However, an early study indicated that there may be transient reticuloendothelial dysfunction during intravascular coagulation initiated by thrombin that may affect clearance capacity [91]. Indeed Davizon and Lopez discuss the potential for defects in clearance to result in an elevated thrombotic tendency [92], exemplified by the observation that splenectomised patients with immune thrombocytopenia purpura (ITP) have higher levels of plasma MP and shortened activated partial thromboplastin time (aPTT) [97]. It is possible that such a defect may be the result of extraordinarily high levels of TF, resulting from the

combined contributions of cancer- and inflammation-related factors, which may overwhelm the system, resulting in a period of transient phagocytic depression, during which these particles persist in the blood, with continual perturbation of the vascular endothelium [98]. This, further aggravated by venous stasis, prevents activated coagulation factors from being cleared and may give the thrombosis the opportunity to develop.

A homeostatic threshold?

The model of Virchow's triad encapsulates the multifactorial pathogenesis of VTE, where any single risk factor or abnormality in any one of the triad components (i.e. venous stasis, endothelial injury or hypercoagulability) can predispose to, but is not sufficient on its own to trigger thrombosis. While it is not necessary to acquire deficiency in all recognised factors, it is likely to be the specific interaction of several of these factors together that will cumulatively result in VTE. More recently, Blix and colleagues have discussed the concept of a "thrombosis potential"; a certain threshold for venous thrombosis reached only by a combination of contributory factors [47]. In accordance with this, it was noted that a high WBC count in isolation did not precipitate thrombosis, but did so in the context of malignancy [47], and Demers *et al*. make comment that, while neutrophil activation and NET generation are likely very important players in cancer-associated VTE, they are unlikely to be sufficient on their own [69], and may only provoke DVT in the context of an additional predisposing factor, such as high levels of TF-MPs. Similarly, high levels of TF-MPs by themselves, even in the context of malignancy, do not cross the 'thrombosis potential' in the majority of cancer patients. Interestingly the contact pathway, which is activated by NETs, is thought to participate significantly during thrombosis, but is considered dispensable for physiological haemostasis [99], emphasizing the importance of leukocytes, particularly neutrophils, perhaps in concentrating key proteins to stabilize the thrombus [12].

The importance of the TF pathway in the initiation and propagation of VTE is undeniable, but it is possible that its significance may differ between cancer types. Certainly in pancreatic cancer, increased levels of TF-MPs do appear to be a key contributory factor, whereas their association with VTE in other cancer types is still a matter of debate. We propose a further putative mechanism for the development of VTE which, this taken into account, may be of particular relevance to pancreatic cancer. We suggest that, in the context of an already hypercoagulable state, inflammation may provide the trigger to drive the procoagulant response. The levels of TF expression still remain a key factor, and it is perhaps the additional effect of inflammation in enhancing leukocyte- and vascular TF expression that invokes optimal thrombus growth [22], accumulating to sufficient concentrations to overcome the physiological threshold set by the *in vivo* anticoagulant mechanisms [35], which themselves are further hampered under inflammatory conditions. In this sense, inflammation produces effects targeting all three aspects of Virchow's triad. Venous stasis is known to promote cell-cell and MP-cell interactions which, in the context of increased circulating TF-MPs and increased leukocyte infiltration, serves to amplify procoagulant and proinflammatory effects. Inflammatory mediators also serve to exacerbate endothelial activation and enhance endothelial perturbation, increasing expression of endothelial TF via PAR1 and PAR2, inducing apoptosis, potentiating MP release and further shifting the haemostatic balance from anti- to pro-thrombotic. Furthermore, innate hypercoagulability is exacerbated by the pronounced change in the systemic balance of procoagulant and anticoagulant factors elicited by inflammatory mediators. In particular, the induction of further TF expression may contribute to levels which are sufficiently high enough to, in the absence of effective counteracting effects, overwhelm natural homeostatic mechanisms. For example, in the setting of inflammatory conditions, such as sepsis and endotoxaemia, which can induce a hypercoagulable state, acute DIC only results when regulatory mechanisms are overwhelmed [100]. In a similar way, the high levels of TF observed when cancer and inflammation combine, may temporarily overwhelm the natural clearance

system, leading to accumulation of TF-MPs and coagulation products, serving to prolong their prothrombotic effect *in vivo* [35].

This hypothesis is illustrated in Figure 1. An innate haemostatic homeostasis is maintained under normal physiological conditions (Fig. 1.a.), where the presence of small amounts of TF in the form of TF-MPs are easily counteracted by swift activation of anticoagulant mechanisms and rapid clearance from the blood system via the endothelium and the mononuclear phagocyte system. Similarly, in a cancer setting, but in the absence of inflammation (or any other instigating factor for endothelial disruption) (Fig. 1.b.), the levels of TF-MPs are likely raised, but are still controlled by innate mechanisms and are not sufficient to exceed the thrombotic threshold and the homeostasis is maintained. In contrast, the addition of inflammation (Fig 1.c.) provides an additional TF stimulus via increased leukocyte infiltration and increased endothelial expression via activation of PAR receptors, and the induction of endothelial apoptosis due to accumulating levels of TF can result in exposure of procoagulant subendothelial layers (Fig. 1.c.ii.). The mechanisms maintaining TF-MP homeostasis may be overwhelmed (Fig. 1.c.i.); anticoagulant mechanisms no longer compensate for the increase and a transient depression of clearance processes (by an as yet undetermined mechanism) allow the threshold to be reached and thrombosis formation to occur.

The interplay of all these processes may explain why the hospital seems to operate as such an 'incubator' of VTE; either through necessity (infection, obstruction, perforation, fracture) or design (planned intervention, surgery, implantation of device, chemotherapy), the precarious 'chronic' yet 'contained through homeostatic mechanisms' cancer coagulopathy is tipped to produce a clinically overt thrombotic event. Although this event may phenotypically come together as a thrombosis, very similarly to the way in which the coagulation cascade comes together to form thrombin, the actual constituent thrombo-anomalies may be very different from cancer to cancer and case to case.

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