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# Microparticles in vascular disorders: How tissue factor-exposing vesicles contribute to pathology and physiology

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ABSTRACT

Coagulation is initiated by tissue factor (TF). Coagulant TF is constitutively expressed by extravascular cells, but there is increasing evidence that TF can also be present within the blood, in particular during pathological conditions. Such TF is exposed on circulating cell-derived vesicles, and its presence has been associated with development of disseminated intravascular coagulation and venous thrombosis. For example, the presence of TF-exposing vesicles in the blood of cancer patients may be associated with their high risk of developing venous thromboembolism. Remarkably, high levels of coagulant TF-exposing vesicles are present in other body fluids such as saliva and urine of healthy persons, suggesting that these vesicles play a physiological role. We postulate that the presence of TF-exposing vesicles in body fluids as saliva and urine provides an additional source of coagulant TF that promotes coagulation, thereby reducing blood loss and contributing to host defence by reducing the risk of microorganisms entering the "milieu intérieur".



## Introduction

Body fluids such as blood contain not only cells but also more than 10 billion vesicles per mL. These vesicles, i.e. microparticles and exosomes, are spherical particles enclosed by a phospholipid bilayer which are released by cells. The diameter of extracellular vesicles ranges between 30 nm and 1 μm. Vesicles have gained a strong clinical interest because they have many functions, e.g. they initiate blood clotting and promote inflammation, facilitate intercellular communication, modulate the immune system, protect cells from waste accumulation and contribute to (multi) drug resistance. Moreover, vesicles differ in concentration, composition and function in diseases as cancer, diabetes and cardiovascular disease compared to healthy subjects, and therefore vesicles have been evaluated for their use as biomarkers [1]. At present, research in the field of cell-derived vesicles is developing rapidly. In this brief overview, we will provide an overview on the role of tissue factor (TF) exposing vesicles in thrombosis and host defence.

## Vesicles and thrombosis

The mechanisms by which cell-derived vesicles contribute to coagulation and thrombosis were elucidated already in the early 1980's. A pioneering study of Dvorak and co-workers showed that several cancer cell lines release a coagulant activity that is associated with membrane vesicles. They postulated that these vesicles (i) provide a phospholipid surface to allow assembly of tenase and prothrombinase complexes, and (ii) exhibit a 'thromboplastin-like' activity, i.e. a tissue factor activity [2]. Later studies confirmed both hypotheses [3,4]. Thus, TF does not only have an extravascular distribution, but can also be present within the blood where TF is associated with cell-derived vesicles [5–8]. Injection of TF-exposing vesicles in animals triggers (TF-dependent) thrombus formation, illustrating that such vesicles are indeed highly procoagulant in vivo [9]. There are four remarks to be made. First, TF exposed on circulating vesicles in human blood is not necessarily coagulant. For example, plasma from patients with diabetes type II contains elevated levels of vesicles exposing TF compared to controls, but these vesicles do not induce TF-dependent coagulation in vitro [10]. Second, coagulant TF-exposing vesicles are removed from circulating human blood by extremely efficient clearance mechanisms. When wound blood of patients undergoing open heart surgery, which contains high numbers of coagulant TF-exposing vesicles, is returned into the systemic circulation, the coagulant activity that is associated with these vesicles is removed from the systemic blood within 20 to 30 minutes [11]. Third, TF-exposing vesicles may interact with platelets adhered to a site of vascular injury, thereby delivering coagulant TF "on the spot" [12].

## Cancer: TF, vesicles, and thrombosis?

Venous thromboembolism (VTE) is a common complication in cancer patients and the second leading cause of in-hospital mortality [13]. The procoagulant phenotype is characterised by increased

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plasma concentrations of activated (coagulation) factor VII and TF compared to controls, indicating activation of extrinsic (TF-initiated) coagulation [14]. This TF may originate from the tumour, because different types of cancer cells express and produce TF [15,16]. This TF is at least in part released on vesicles [2–4], which in turn may enter the blood [17]. Further evidence that such vesicles may contribute to VTE comes from findings that (i) plasma from cancer patients contains increased numbers of TF-exposing vesicles (compared to controls) [18,19], (ii) the TF coagulant activity that is associated with vesicles is elevated in cancer patients [20,21], (iii) cancer patients who developed VTE have higher numbers of TF-exposing vesicles and an elevated TF coagulant activity associated with these vesicles than cancer patients without VTE [22–25].

Although VTE is clearly a complication occurring in many cancer patients, treating all cancer patients with prophylactic doses of anti-coagulant therapy to prevent VTE is unattractive due to the risk of bleeding. Therefore, in 2010 we have initiated a study to investigate whether cancer patients at risk of developing VTE can be identified. We hypothesize that such high risk patients have elevated numbers of coagulant TF-exposing vesicles already *before* the onset of VTE. In this study, blood is collected from cancer patients before a new gift of chemotherapy, and we perform a vesicle-based coagulation assay, a plasma recalcification test in which the clotting time dependent on both phosphatidylserine and TF [21,26]. Our pilot data confirmed our hypothesis [21], and this study is now ongoing in eight hospitals and more than 600 patients have already been included.

Recently, Wang and co-workers showed in nude mice that although two human pancreatic tumour cell lines produce comparable numbers of TF-exposing vesicles, only one cell line produced detectable levels of vesicles-associated TF coagulant activity, implicating that there is no direct relationship between MP-associated TF antigen and activity [27]. Thus, the relationship between the presence of TF-exposing vesicles in plasma and the TF-dependent coagulant activity of vesicles in cancer patients is unclear. This is confirmed by a nearly completed study in Amsterdam, in which we measured both the presence of MP-associated TF antigen and TF coagulant activity in plasma samples collected from over 200 cancer patients. We found no association between TF antigen and activity. For example, plasma samples clearly containing a TF coagulant activity contained no detectable numbers of vesicles exposing TF (A. Kleinjan, manuscript in prep.), suggesting that either minute quantities of coagulant TF are sufficient to trigger coagulation, and/or that TF is associated with vesicles with a diameter too small to be detected by flow cytometry [28].

To which extent coagulant TF-exposing vesicles that are present in plasma of cancer patients originate from the tumour is unclear. We determined the cellular origin in cancer patients who had numbers of TF-exposing vesicles above the >95th percentile in the before mentioned study. In 5 patients, TF-exposing vesicles stained for both tumour markers and markers of blood cells, and in these patients more than 100% of the vesicles were labelled, indicating fusion of vesicles or transfer of TF between vesicles and/or cells. In 8 other patients, however, only 30% of the vesicles exposed blood cell antigens but no detectable levels of tumour antigens, and the cellular origin of the other 70% of TF-exposing vesicles could not be established (A. Kleinjan, manuscript in prep.). Thus, to which extent coagulant TF that is associated with circulating vesicles in blood of cancer patients originates from the tumour remains unclear. The fact that plasma samples containing the highest levels of TF coagulant activity contain no detectable levels of TF-exposing vesicles clearly illustrates that additional studies will be essential to elucidate the cellular origin of vesicles exposing coagulant TF in cancer patients.

## TF-exposing vesicles in body fluids other than blood

In essence, coagulation and inflammation are processes that contribute to host defence. Coagulation contributes to haemostasis, thus reducing blood loss and minimizing the risk of microorganisms entering the “milieu intérieur”. Under normal, physiological conditions virtually no coagualant TF is associated with circulating vesicles. When such TF is present, this TF is associated with e.g. bleeding as in disseminated intravascular coagulation, or with VTE as in cancer patients. Recently, we showed that saliva of healthy humans subjects contains high levels of vesicles exposing coagulant TF that is capable of triggering the clotting of blood [26]. We therefore hypothesized that the reflex of humans and animals to lick a wound may – at least in part – be explained by the presence of such vesicles in saliva. Because also urine from healthy human subjects contains a clearly detectable TF coagulant activity that is associated with vesicles, we postulate that the presence of such vesicles in body fluids which are in contact with the epithelium and the “milieu exteriéur” may be associated with haemostasis, host defence and thus contribute to maintenance and survival of an organism.

## Conclusion

The presence of vesicles exposing coagulant TF depends on the type of body fluid studied, and whether the fluid is collected from healthy persons or from patients. Whereas vesicles exposing coagulant TF in saliva and urine are likely to contribute to host defence, i.e. protection of the “milieu interiéur”, the presence of such vesicles in blood seems to be associated with disease or disease progression.

## Conflict of interest statement

There is no conflict of interest for these authors.

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