Tissue factor

Biological function and clinical significance

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ABSTRACT

Tissue Factor is the principal cellular initiator of normal blood coagulation. It is frequently encrypted in the plasma membrane of cells in contact with blood, but under certain pathological conditions endothelial cells, monocytes or macrophages may express tissue factor; and hence trigger coagulation activation. Aberrant expression of tissue factor by these cells is thought to be responsible for the thrombophilia found in septic shock, atherosclerosis and cancer. Tissue factor is produced by tumor-associated macrophages where it is believed to play an important role in tumor growth and dissemination. It may also be involved in other cellular processes such as intracellular signalling, angiogenesis and embryonic blood-vessel development. Tissue factor can be found both as free (soluble tissue factor) and membrane bound forms. Several studies have shown that measurements of any of these forms may provide clinically significant information, particularly in patients with malignant and inflammatory diseases, and are cost-effective.

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Various human tissues,[1] activated vascular endothelial cells, blood monocytes or tissue macrophages, and tumor cells,[2-4] have all been shown to express a potent initiator of blood coagulation that does not normally circulate in the blood.[5] Biological fluids have also been shown to contain similar procoagulant activity (PCA), by both functional and immunologic criteria.[6,7] This coagulant is commonly referred to as thromboplastin or coagulation factor III, but it is perhaps best known as Tissue Factor (TF) or more recently, cluster of differentiation (CD)142.

Tissue factor apoprotein is a 43,000-45,000 MW, single-chain, integral plasma membrane glycoprotein with no intrinsic protease activity.[8,9] It serves as a receptor and essential co-factor for the serine protease blood coagulation factors VII and VIIa.[10] Unlike other co-factors (V and VIII), no proteolytic modifications are involved in the association between TF and factor VII/VIIa; hence coagulation is initiated only by physical association between TF and either factor VII/VIIa.[11] The interaction between TF and VII/VIIa is, however, accelerated by acidic phospholipid such as phosphatidylserine.[12] The bimolecular complex (TF:VII:VIIa) activates factors IX and X by limited proteolysis, in the presence of Ca^{++}. This triggers downstream coagulation pathways, which eventually lead to thrombin generation and fibrin formation **(Figure 1).**

The amino acid sequence of the mature protein suggested that TF is comprised of 3 distinct domains, an extracellular domain (residues 1-218 or 219) also called free or soluble TF (sTF), a membrane-spanning hydrophobic structure (residues 220-242), and a cytoplasmic region, residues 243-263 **(Figure 2).** Purification studies have revealed that TF is a novel protein, lacking significant homology either with the vitamin K dependent coagulation factors or with other known proteins.[13] However, the 21 amino acids on the C-terminus of the cytoplasmic domain resemble the same region of other membrane proteins such as thrombomodulin and the low density lipoprotein receptor.[14] Several pharmacological

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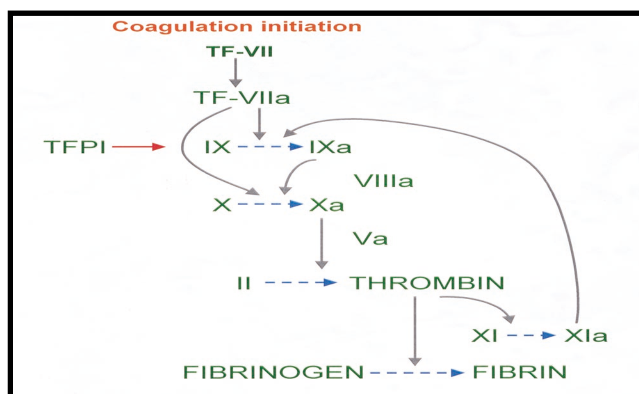

Figure 1 - The current concept of the coagulation process. TF=Tissue Factor, VIIa=7a, TFPI=Tissue Factor Pathway Inhibitor, IX=9, IXa=9a, X=10, Xa=10a, II=2, XI=11, XIa=11a.

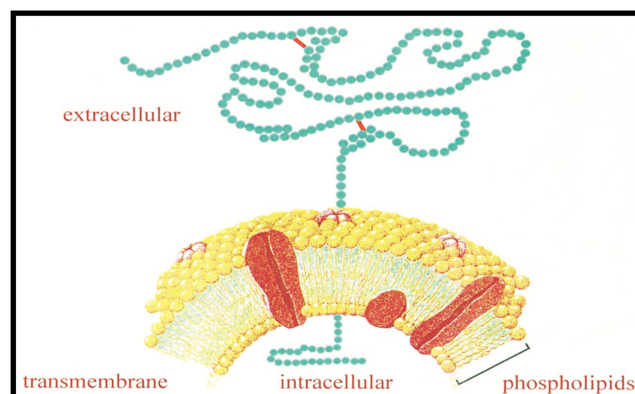

Figure 2 - Model of tissue factor.

agents and inhibitors can significantly influence or modulate TF production in both intact and cultured cells.[15] Tissue factor expression in cultured monolayer cells can be dependent on cell adhesion and spreading. There is considerable evidence to suggest that TF activity in cultured cells is due to cell surface expression.[16] Repeated washing of intact cells and trypsinization of cultured cells, at high concentrations of trypsin, were shown to reduce TF activity.[15] Tissue factor activity was also increased by freeze-thaw lysis in several cells, which suggest that TF activity on the surface of cultured cells is present in cryptic form.[15] Generally, TF appears to be produced de novo, rather than being stored intracellularly.[17]

This review, will discuss some of the aspects of this interesting molecule, focussing mainly on its biological function and clinical significance.

Forms of tissue factor. Two forms of TF have been described; free (soluble) or membrane bound. The latter includes both cellular (such as those produced by monocytes, macrophage, endothelial or tumor cells) and lipid-vesicle bound (such as those found in urine or in semen).

Soluble. Plasma TF (pTF) is the sTF form and results from proteolytic cleavage at or near the linkage between the trans-membrane and the intracellular domains.[18] The nature of the protein (membrane-bound) suggests that TF would not be found free in the plasma. However, it has been shown that the extracelluar domain of the protein may appear in the plasma where it can be detected both antigenically using specific enzyme-linked immunosorbent assay (ELISA)[6] and functionally using a factor VII dependent chromogenic assay.[7]

Membrane bound. Cellular. Monocytes, macrophages, endothelial and tumor cells have long been known to expresses TF. Tissue factor expression by monocytes and tumor cells is dependent on activation state reflected by alteration in expression of phosphotidylserine, as detected by the binding of Annexin-V, a human placental anticoagulant protein.[19] Increased monocyte TF (mTF) expression is of particular importance in disease status as various stimulators can modulate mTF expression, both in vivo and in vitro, the same applies to tissue macrophages.[3] The co-operation of lipoproteins with different degrees of effectiveness, lymphocytes, platelets or platelet derived membranes and granulocytes have all been reported to augment mTF activity in the presence of lipopolysaccharide (LPS).[20-22] Monocytes and macrophages are thought to express TF as one of several ways they participate in host defence against tumor cells.[23] The process of monocyte activation is complex, involving multiple pathways, and is yet to be fully elucidated. This and other aspects of mTF have been recently reviewed elsewhere.[24]

Lipid-vesicles bound. 1. Urine. Urine has for many years been known to contain a powerful PCA,[25] which was first thought to be a tissue thromboplastin-related substance.[26] It was later found that urinary PCA catalysed the conversion of prothrombin to thrombin solely in the presence of platelet factor 3, factor V and calcium ions - a prothrombinase type of activity, and was therefore classified as 'platelet co-factor' rather than TF.[27] However, TF does not require the addition of platelet factor 3 to activate prothrombin, although its addition will substantially enhance TF's activity. This assay system, therefore, will not detect true tissue thromboplastin, it probably measures a prothrombinase-like activity.

Subsequently, a factor in urine that has the properties of TF has been described. Kurosawa et al,[28] purified the procoagulant and demonstrated its promotion of clot formation in a factor VII-dependent manner. Aoki and von Kaulla[29] suggested that the urinary PCA is raised in small particles and they report a factor with the properties of "prothrombinase". Later Wiggins et al,[30] showed that urinary PCA was raised in lipid-associated vesicles and was mainly factor VII-dependent as assessed by

clotting assays in human factor VII-deficient plasma. Thus the procoagulant can activate factor X in the presence of factor VII and calcium and, like TF.[31] is inhibited by conconavalin A. Carty et al,[32] confirmed that the urinary procoagulant was TF (Urinary TF, uTF) by demonstrating almost total inhibition of the activity by a specific antibody to human TF. An estimate of uTF molecular weight in our laboratory using western blotting revealed that uTF has a similar molecular weight to that of TF derived from other human tissues, of 43,000.[32] The clinical potential for uTF has been reviewed elsewhere.[33]

2. Semen. Human semen contains enzymes and inhibitors of the hemostatic system,[34] that may have roles in the clotting and fibrinolytic process. Although a limited number of studies have addressed the potential role of these enzymes and inhibitors,[35,36] the role of blood-coagulation factors in semen remains speculative. It has previously been reported that semen contains TF antigen.[6] More recently the presence of both TF and factor VII in human semen has been independently confirmed[37] and it has been suggested that seminal TF originates from the prostate and is associated with prostosomes,[37] a vesicular product secreted by acinar cells of the prostate gland.[38] Tissue factor in semen may serve to limit bleeding and consequent vascular access by semen-born agents as a result of tissue damage during intercourse and may also contribute to the anti-inflammatory properties attributed to prostosomes.[37,39] However, TF function in semen appears to be not related to its role in blood coagulation, as there is an absence of factor X. In addition, there is a limited amount of factor VII in semen, which is present at picmolar concentrations.[40] It is therefore possible that TF in semen may have entirely different actions, which are as yet largely unidentified. Evaluation of semen TF in infertile patients showed a 16-fold variation in TF-VII activity and no relationship was found between TF and number of days of abstinence before sampling, specimen pH, sperm counts or sperm motility.[40]

Methods for tissue factor measurements. Progress in the understanding of the molecular structure and the biochemistry of TF have led to increased interest in measuring aspects of TF activity and antigenicity in "normal" and "disease states". Thus, several sophisticated laboratory methods at reasonable cost have become available for TF assessment, both on cells and other biological materials, with substantial clinical potential. These include TF measurements on the surface of blood monocytes, macrophages, tumor cells, and tissue exudates, and in various biological fluids. Measurements are currently being performed using 2 types of assay, functional and immunological.

As stated previously, pTF can be measured using a commercially available ELISA assay[6] or a chromogenic assay.[7] Although ELISA assays are currently used for the measurement of pTF antigen, the interpretation of the results is complicated. This is due to uncertainty as to whether the detectable, free pTF is in a functional form and if it represents the status of clotting activation. In addition, there is no well recognised form of human sTF for valid standardisation and cross-referencing purposes that can be used between laboratories.

There has been substantial interest in measuring mTF PCA, however there are concerns over interpretation due to the diverse methodologies used.[41] While some have used clotting techniques to quantitate TF activity,[42] others have utilized chromogenic assays.[43] Functional approaches have employed several methods for the isolation of monocyte populations, many of which may artifactually affect results.[44] This poses additional problems since a few workers measure TF on disrupted,[45] rather than intact cells.[46] Numerical expression of mTF adds complexity to the subject. Spillert et al,[47] used whole blood re-calcification time as a measure for mTF activity. The technique has the advantage of being simple, with minimal requirements in terms of both equipment and expertise, and it also automatically takes into account the potential roles of other cells such as platelets and granulocytes[48] in the response of mTF to endotoxin. Its major disadvantage though, is that the assay will not give information on isolated baseline cells, hence this assay requires further refinement to provide information on both baseline and stimulated TF expression.[41]

Antigenic approaches for measuring mTF by means of flow cytometry are now widely used.[46,49] The limitation to these approaches is that other cells may influence measurements. This is particularly impoprtant when a population of stimulated monocyte cells are studied, since assessing the coagulation process in whole blood may be physiologically more relevant than assaying isolated hemostatic components.[50] A limited number of studies have employed a 2 stage chromogenic substrate assay in the investigation of uTF PCA,[32] however, this assay may not have been completely reliable. Recently a novel one stage kinetic chromogenic assay for uTF measurements has been described.[7] An antigenic approach using an ELISA assay has also been employed for uTF measruement.[6] Tissue factor detected by this technique may or may not be functional.

Factors that might influence tissue factor measurements. There may be multiple variables that influence the measurement of blood coagulation parameters and TF in particular, but the most obvious are age, sex, drugs and cigarette smoking. Other factors that are specific to mTF measurements are reviewed elsewhere.[24]

Age. Calmus and Robert[51] reported an increase in mTF activity with age. Its levels were dramatically increased in postmenopausal women.[52] However, several independent studies have shown that, neither

baseline nor stimulated mTF level in vitro was significantly affected by age and no functional or immunological association in mTF level was found between the 2.[46,53] Similarly, no significant difference was observed for uTF[7] or pTF levels.[54] These are important findings if this analyte is to find a place in clinical practice.

Gender. There is no evidence in the literature to suggest that gender might play a major role in TF activation, although females were noted to have faster rates of whole blood clotting.[50] Subsequent studies in our laboratory have, however, reported a lack of correlation between mTF or uTF and gender.[24] Similarly pTF showed no sex related differences.[54]

Drugs. Several anti-inflammatory drugs have been shown to decrease mTF activity, these include corticosteroids,[55] methylprednisone[55] dexamethasone.[56] In contrast cyclosporin, cisplatin and adriamycin have been reported to enhance mTF activity.[57] The regulation of TF by these agents might be clinically significant in thrombotic, fibrinolytic, or both, problems associated with chemotherapy. Five percent of women receiving chemotherapy following mastectomy for breast carcinoma developed serious thrombosis[58] and many more women receiving tamoxifen as post-surgical treatment for the same disorder developed venous thrombosis or disseminated intravascular coagulation (DIC) compared to controls.[59]

Smoking. Cigarette smokers showed more rapid heparin elimination rates than non smokers,[60] and tobacco materials were found to shorten the partial thromboplastin time and euglobulin clot lysis and generate factor XII and kinin activities.[61] Heavy cigarette smokers also had higher total leukocyte counts than non-smokers.[62] While pre-menopausal women who use the oral contraceptive or smoke may have enhanced mTF gene transcription, which could be hormonal dependent,[63] both mTF and uTF activity were subsequently found to be not significantly affected by cigarette smoking.[24]

The clinical significance of tissue factor. As the principal biological initiator of blood coagulation, TF is believed to play a critical role in thrombosis and thrombogenesis.[10,64,65] Tissue factor expression by monocytes/macrophages and endothelial cells can be up-regulated by endogenous inflammatory mediators including interleukin 1, tumor necrosis factor, vascular permeability factor (VPF), complement, LPS, plasma lipoproteins, plasma protein, collagen, as well as immune complexes and other physiological and pathological mediators.[23,55,56,65,66-70]

Increased antigenic TF levels in interventional cardiologic procedures such as percutaneous transluminal coronary angioplasty as a result of endovascular injury.[6] The detection of TF antigen in senile plaques of Alzheimer's disease and in atherosclerotic plaques.[71] The implication of TF in atherogenesis[72] and the ability of atherosclerosis to induce thrombosis[65] are all suggestive of the clinical and pathological importance of TF. Tissue factor is present in large quantities in nervous tissues and cerebrospinal fluid (CSF) in particular,[73] thus, its measurement may be clinically important in disorders of the central or spinal track such as, thrombotic stroke, meningitis or spinal injuries. Indeed Fareed et al,[6] in their pilot studies on randomly selected CSF samples showed high TF values, although clinical correlation with the TF level remains to be established.

Clinical studies in hemophiliacs unresponsive to replacement therapy because of inhibitors have shown that infusion of factor VIIa can stop bleeding.[74] Such responses with enhancement of TF-dependent activation of coagulation further emphasize the clinical importance of TF.

Tissue factor may also play an integral role in tumor dissemination and growth. It has been shown that TF expression promotes hematogenous tumor dissemination,[75] controls the balance of angiogenic and anti-angiogenic factors and is critical for the growth and progression of solid malignancy.[76-78] The relationship between neovascularisation, angiogenesis and angiogenic proteins is not only important in neoplastic diseases but also in non-neoplastic conditions. Overexpression of TF in wounds modulates wound healing by stimulating neovascularisation into the wounded area.[79] This suggests that TF might be useful as an antitumor therapy against hypervascular tumors or as an agent against delayed wound healing. In addition, it has been suggested that TF may serve as an important morphogenic factor during embryogenesis.[80]

In the present review, I will briefly address the potential role of TF in relation to tumor-associated angiogenesis, cancer and glomerulonephritis.

Tissue factor and tumor-associated angiogenesis. Tumour neovascularisation and angiogenesis is an important feature in tumor growth and biology.[81] Of a particular interest in this area is TF expression by vascular endothelial cells in tumor beds which suggests a relationship between the angiogenic regulation and the hemostatic system that may be more than co-incidental. For example: 1. Transfection with a TF cDNA sense construct, creates tumor cells that overexpress TF and increased vascular endothelial growth factor (VEGF) production by 1000-fold.[82] Both TF and VEGF are expressed by vascular endothelial cells and tumor cells in malignant breast tissues but not their benign counterparts[78] and their activity in melanoma cells was strongly correlated.[82] 2. Tumour cells over expressing TF demonstrated increased mitogenic activity for endothelial cells in parallel with enhanced transcription of VPF/VEGF, and diminished transcription of thrombospondin, an inhibitor of angiogeniesis.[82] 3. Vascular permeability factor in murine fibrosarcoma can induce TF expression in

endothelial cells and monocytes and promotes monocyte migration into the tumor bed where it alters the properties of the vessel wall and enhances coagulation activation.[69] When a murine fibrosarcoma was stably transfected with mouse TF cDNA, its vascularisation in vivo was significantly enhanced, whereas TF-anti-sense suppressed tumor vascularisation and growth.[83] 4. Tumour cells may also recruit host cells such as macrophages, which in addition to TF expression may produce their own angiogenic proteins.[84,85] 5. Tissue factor on the tumor cells and on the endothelium of tumor-induced microvessels can cause thrombin formation that can then generate fibrin within the tumor.[78,86] A significant association was found between TF expression and microvessel density (MVD) which is related to patient survival time:[87] Tissue factor negative carcinomas more frequently exhibited low MVD.[88] 6. Thrombin can also cleave osteopontin,[89] which is over-expressed in human carcinoma.[81] Both fibrin and osteopontin have been shown to participate in the migration of endothelial cells[89] and to potentiate angiogenesis.[81,90] Plasma derived fibrinogen, escaped from leaky neovasclature, complexes with the platelet-tumor cell aggregates (fibrin gel) having been converted to fibrin. This "provisional stroma" is ultimately replaced by granulation tissue and mature stroma. Inflammatory cell infiltration into the tumor is minimized as a result of fibrin deposition. Thus the efficiency of blood vessel growth, tumor growth and metastasis is enhanced.[91]

Tissue factor and cancer. Local or systemic initiation of blood coagulation can be triggered by a tumor or related products. Tumour cells can directly interact with platelets,[92] produce procoagulants such as Cancer Procoagulant (CP) which directly activates factor X[93] or TF, or both, which requires factors VII/VIIa for its proteolytic activity on factor X to be accomplished. Tissue factor expression can also be induced in monocytes/macrophages and endothelial cells in response to a direct stimulation by tumor products (antigens, protease, etc), or indirectly following activation of other components of the immune system such as T lymphocytes.[94,95]

Extracts of tumor tissue have been shown to shorten the plasma re-calcification time more than most normal tissue[96] and circulating mononuclear cells from tumor-bearing animals have significantly more TF activity than those from control animals.[97] Patients with cancer have significantly higher TF levels compared with normal controls[24,33,46,53] and cultured monocytes from cancer patients express increased amounts of TF compared to normal subjects.[3] Dasmahapatra et al,[98] using a whole blood re-calcification time showed an increased level of LPS-induced mTF in patients with various tumors and suggested that this could be used to distinguish with some certainty patients with malignant diseases from those with benign non-inflammatory diseases. Contrino et al,[78] demonstrated using both immnohistochemical technique and a novel probe for functional TF, that TF expression by endothelial cells is present only in the blood vessels of malignant breast lesions but not in the vessels of benign breast lesions. Most recently, Ueno et al,[99] suggested that TF expression in breast cancer tissue may be of predictive value in prognosis and distant metastasis. Thus these studies demonstrate an association between TF expression and the malignant phenotype, although the TF detected is on host cells either circulating or embedded in the tumor stroma.

Tumor cells can cause platelet aggregation in vitro and thrombocytopenia in mice when injected intravenously.[100] In addition, platelet counts seem to be positively associated with the development of metastatic disease and thrombocytopenic animals did not develop metastases.[101] Fifty-seven percent of patients with cancer not receiving chemotherapy exhibited thrombocytosis.[102] An earlier review of the subject[94] quotes values between 30% and 60%. Platelet counts in chemotherapy treated cancer patients tend to increase before death.[103] Excessive platelet numbers may stimulate mTF expression in vitro[53] suggesting a possible interaction between monocytes and platelets in vivo impinging on thromboembolic complications in patients with cancer. Furthermore, unstimulated mTF levels in patients with advanced lung cancer were found to be linearly correlated with other markers of in vivo coagulation activation such as Fibrinopeptide A (FpA) and also tumor burden.[3,104,105] Both in vivo and in vitro LPS-stimulated mTF levels also positively correlated with those of the plasma levels of the cross-linked fibrin degradation product (D-dimer or D-D).[106] The D-D levels are increased in patients with cancer and levels may reflect tumor progression.[107] This provides further evidence for coagulation activation and subsequent fibrinolysis, suggesting that increased fibrin formation in vivo is at least partly dependent on spontaneous mTF expression.

Both uTF and mTF levels showed an association with increasing tumor grade or stage and with other conventional markers of tumor progression.[24,33] Levels were also significantly higher in patients with recurrent bladder malignancy compared to those with a normal check cystoscopy,[24,33] and increased in patients who subsequently died.[24,33,53,108]

Leukemic cells express thromboplastic activity and LPS-stimulated monocytes from patients with Hodgkin's disease, those with relapsed Hodgkin's disease and non-Hodgkin lymphoma had significantly raised mTF PCA.[109,110] Monocyte tissue factor activity was also elevated in patients with acute monoblastic and chronic myelomonocytic leukaemias.[111]

Increased mTF expression post-operatively is also a common finding. Increased mTF activity was observed after surgery,[112] between the first and 5th days post-operatively.[113] It has also been suggested that mTF activity increasing one day after "curative" surgery for malignant tumors could be taken as an indication of metastasis.[45]

Tissue factor and glomerulonephritis. Activation of blood coagulation and glomerular fibrin deposition (GFD) have been associated with some forms of inflammatory glomerular diseases in man and laboratory animals. Fibrin deposition within and around the glomerulus has been observed by several investigators[114-116] and is thought to play a major pathogenetic role in the development and the progression of the disease.[117] The underlying biological mechanisms of such phenomena are complex, but the TF-dependent pathway has been implicated.[118] However, it remains to be established the cell type responsible for GFD within the nephron. Tissue factor is also found in a lipid-associated form in urine where its level was slightly increased in the urine of patients with Glomerulonephritis (GN) and serum creatinine levels higher than 1.5 mg/dl compared to those with lower levels and normal controls.[119] In a subsequent study, our group showed that uTF excretion in patients with GN were significantly higher than those of controls or patients with renal stones and no evidence of inflammation (erythrocyte sedimentation rate (ESR) normal).[120] In addition we found increased uTF levels in immune complex GN compared to non-immune complex forms and suggested that elevated uTF levels may reflect the etio-pathogenesis of GN.[120]

In conclusion TF is not only the primary physiological initiator of blood coagulation but may also play a role in the biology of various diseases, particularly solid malignancies, where it potentiates metastasis and angiogenesis, and mediates outside-in signalling. Tissue factor is increased in both tumor-associated macrophages and blood monocytes and has been implicated in abnormal coagulation activation seen in patients with inflammatory conditions and solid malignancies. Tissue factor levels can be measured cost-effectively in various body fluids and on monocytes. Its activation in malignant and inflammatory conditions may have immense clinical potential.

The relationship between the hemostatic system and the pathophysiology of some diseases seems intimate. Although a lot has recently been achieved in our understanding in this area particularly at the molecular levels, many more questions remain to be addressed. For example, in cancer, could changes in TF levels during the course of malignant disease indicate tumor load and perhaps prognosis in individual patients? In this context, longitudinal studies are required to evaluate fully the role of TF as such a marker for tumor progression and as a tool for selecting the most appropriate treatment strategy for malignant disease. In the first instance a large scale study correlating TF levels with pathological findings is required.

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