*Review Article*
# The origin and onset of acute venous thrombus

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**Abstract:** Under the condition of immune cell balancing function collapse, acute venous thrombosis originates from intravenous immune adhesive inflammations triggered by cells which are infected by foreign pathogenic microorganism and malignant cells. With the condition of immune cell balancing function collapse, the human body lost the function of clearing intravenous foreign pathogenic microorganism and malignant cells timely and effectively. Thus, integrins β2 and β3 on the membrane of white blood cells and platelets are activated to combine with the ligand fibrinogen into a reversible mesh-like structure, which is like the intravenous biological filter and acts as physical defense of the human body to prevent the cells which are infected by foreign pathogenic microorganism and malignant cells in the distal veins from flowing back to the whole body. Meanwhile, blood cells mainly red blood cells stagnate and fulfill the filter, which blocks the blood flow in the local veins and thus results in venous thrombotic diseases. People with collapsed immune cell balancing functions are the certain groups of people who will develop venous thromboembolism. Anyone who had venous thromboembolism indicates alloantigen cells in the veins, which are mainly pathogenic microorganism infected cells and malignant cells and trigger the onset of venous thromboembolism. Only under the condition of immune cell balancing function collapse, the risk factors, such as advanced age, infection, trauma, surgery, autoimmune disease, pregnancy as well as long trip syndrome, could cause venous thromboembolism.

**Keywords:** Origin, venous thromboembolism, core protein, immune cell balancing function, infected cell, malignant cell

## Query raised in clinics

Venous thromboembolism (VTE) includes pulmonary thromboembolism (PE) and deep venous thrombosis (DVT). Among them, PE has become a global medical care problem due to its high morbidity, misdiagnosis rate and mortality [1, 2]. VTE can be divided into two categories, genetic VTE and acquired VTE. According to the results of epidemiological investigations, the incidence of genetic VTE is relatively low, while most of the VTEs are acquired VTEs. Both of them can be called symptomatic VTE, when hard to be distinguished [3]. Diseases and constitutional factors that increase the risk of VTE has been identified by organizations such as the American College of Chest Physicians (ACCP) which has published nine editions of their guidelines for VTE diagnosis, treatment and prevention since 1995 to 2012 [3]. Proposed risk factors include advanced age, infection, malignancy, autoimmune disease, surgery, trauma, pregnancy, long trip syndrome,

family history and so on. ACCP has raised the risk stratification of surgical patients. Different measures should be taken in patients with different stratification to prevent VTE. Actually, only a small part of the patients with same risk stratification and same external environment have had VTE, while others do not. In 2008, Shackfore et al. [4] reported that among the 84% of 37619 surgical patients who are partly or totally treated and prevented according to the guideline from 1995 when the first ACCP was published to 2004, the numbers of symptomatic VTE increased instead of decreased, and there is segregation between preventing risk factors and VTE occurrence.

Thus, here the questions come. Why does the incidence of VTE increase as the age increases? Why does the incidence of VTE stay high in patients with malignancies? Why does only a small part of patients with the same infection develop VTE? Sudden death led by surgeries, pregnancy, delivery or long trip syndrome
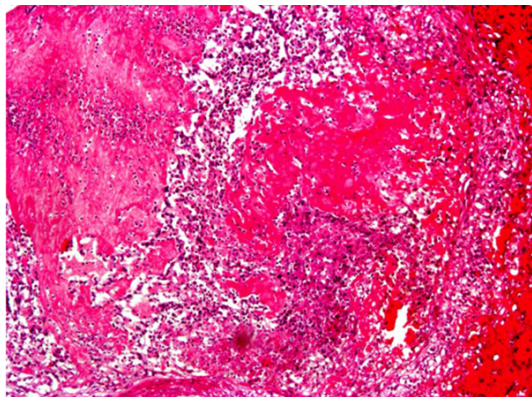
**Figure 1.** HE staining of thrombus showed that venous thrombus was red thrombus in which massive red blood cells and white blood cells with dark-brown nuclei aggregated (HE staining, ×200).

caused acute PE was always hard to prevent. However, the vast majority of the population will not develop VTE in the same conditions. Both belonging to thrombus, acute arterial thrombus is white thrombus, while acute venous thrombus is red thrombus. What does the pathological difference mean? Thrombolytic therapy is effective for arterial thrombosis within several hours after onset, but venous thrombosis, with a wide thrombolytic time window, can be delayed to several days, two weeks, or even longer [5]. What causes the difference in the thrombolytic time window between venous and arterial thrombosis in the same body. Venous thrombosis can autolyze, while arterial thrombosis cannot. For VTE patients, oral anticoagulants are usually recommended for 3, 6 or 12 months and occasionally life-long [6]. Currently, there are no objective criteria for individual evaluation that complicates the selection of anti-coagulation therapy by physicians. Furthermore, even with standard anti-coagulation therapy and INR, some patients still develop CTEPH. Thus, the physicians are extremely puzzled about anticoagulant usage.

Query raised in clinics usually originates from clinical practices. The risk factors of VTE are only the clinical phenomenon of incident VTEs and the summary of evidence-based medicine, not the essence of this disease. So far, the medical resources put into the global prevention of VTE do not have the predicted effects. The reasonable explanation of this separation phenomenon between clinical prevention and treatment guidelines of VTE and clinical prac-

tices is that the etiology and pathogenesis of VTE are still unclear.

*Protein components analysis of acute venous thrombus*

Acute venous thrombosis is red thrombus to naked eyes, which is composed of red blood cells, platelets, white blood cells and plasma proteins under microscope. The acute venous thrombosis taken out from the body was fragile (**Figure 1**).

Mass spectrographic analyses showed that a large majority of proteins were fibrinogen; the remaining proteins included serum albumin and cytoskeletal proteins [5]. The reversible combinations between fibrinogen and their ligand proteins theologically explain that acute venous thrombus is easy to autolyze, delayed thrombolysis is effective, and it is easy to lyse the thrombus through interventional fragmentation. Acute venous thrombosis is red thrombus, which is composed of red blood cells, platelets, white blood cells and fibrinogen. But how does fibrinogen bind to blood cells in the formation of thrombus? MS/MS and bioinformatics analysis of thrombus in patients with acute PE showed that subunits β1, β2 and β3 in integrins were the core proteins of thrombus (**Figure 2**).

Integrins are one of important members in cell adhesion molecule family, and mediate the adhesion between cells and between cells and extracellular matrix (ECM), and are involved in the bidirectional signaling transduction between cells and ECM. Integrins combine to different ligands in various cellular processes, which are physical or pathological process of angiogenesis, invasion, metastasis, inflammation, wound healing and coagulation [7].

Integrin is a transmembrane heterodimer composed of subunits α and β at a ratio of 1:1. To date (**Figure 3A**), a total of 18 α subunits and eight β subunits have been identified and they can form 24 functional heterodimers which may be classified into eight groups (β1-β8) on the basis of β subunit. In the same group, the β subunit is identical, but the α subunit is distinct. At rest, the α subunit is covered by the β subunit and thus the integrin is unable to bind to ligands. Following activation, the extension of the β subunit exposes the α subunit. The α
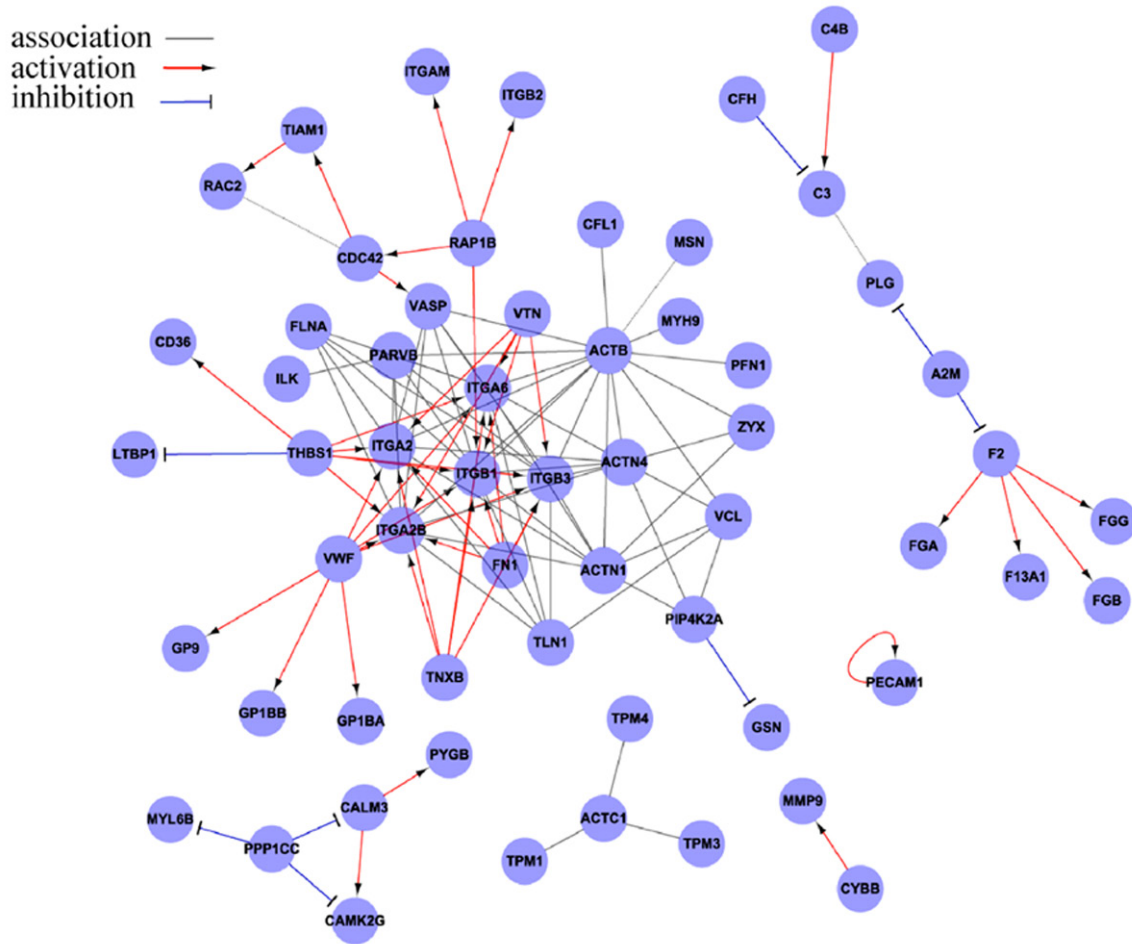
**Figure 2.** MS/MS and bioinformatics analysis of thrombus in patients with acute PE. Integrin subunits β1, β2 and β3 were the core proteins of thrombus.

subunit mainly mediates the specific and reversible binding between integrins and their ligands (**Figure 3B**), and the β subunit dominates the signal transduction and regulation of affinity of integrins [8-10].

*Localization of core proteins in acute venous thrombus*

The β1 subunit is mainly found on the lymphocytes and platelets, and its ligand includes laminin, collagen, thrombospondin, fibronectin and VCAM-1. The β2 subunit is mainly distributed on the neutrophils and monocytes, and its ligand includes fibrinogen, ICAM, factor X and ic3b. The β3 subunit is mainly observed on the platelets, and its ligand includes fibrinogen, fibronectin, vitronectin, vWF and thrombospondin [11-13].

The author [14] collected the thrombi from patients with acute PE, and detected the expression and distribution of integrin β1, β2 and β3 in thrombi and ligands of integrin subunit β1, β2 and β3 by immunohistochemistry. Immunohistochemistry showed dark-brown integrin β1 was expressed on the lymphocytes, but no expression of laminin, fibronectin, collagen-I and collagen-II was observed on the lymphocytes; dark-brown integrin β2 was expressed on the neutrophils, which bound to fibrinogen; The ICAM, factor X and iC3b were expressed on neutrophils; dark-brown integrin β3 was expressed on platelets which aggregated to be thrombotic skeleton and coral-like structure; these platelets bound fibrinogen to construct mesh structure (**Figure 4A**); No expression of fibronectin, vitronectin or vWF was observed on the platelets; dark-brown fac-
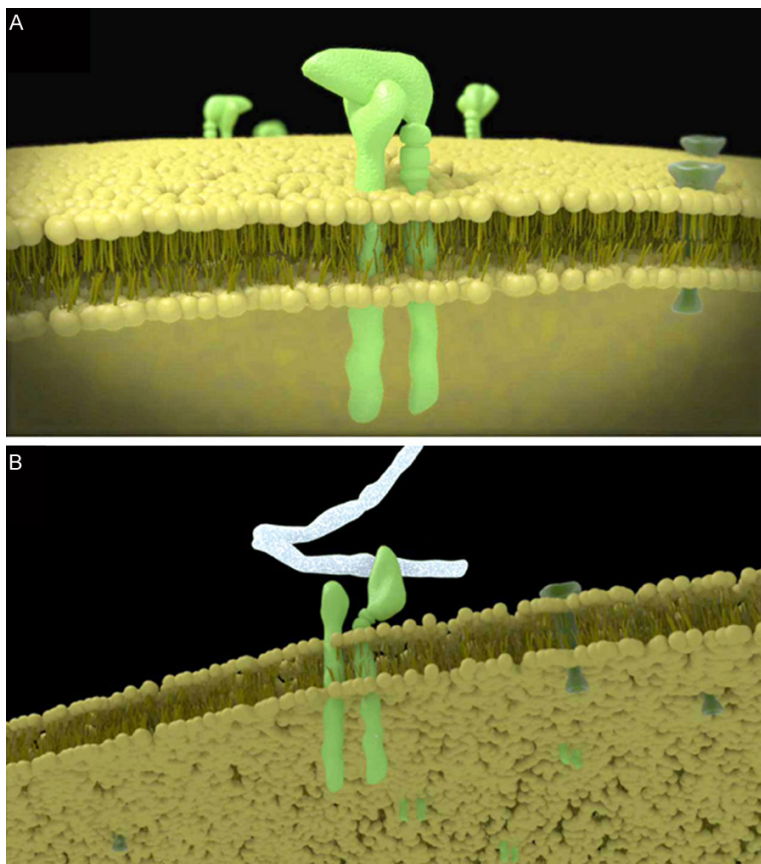
**Figure 3.** A. Mode pattern of the integrin on the cell membrane. Integrin is a transmembrane heterodimer composed of subunits α and β at a ratio of 1:1. At rest, the α subunit is covered by the β subunit and thus the integrin is unable to bind to ligands. B. Following activation, the extension of the β subunit exposes the α subunit. The α subunit mainly mediates the specific and reversible binding between integrins and their ligands.

the body regulation and the construction of biological filter?

The author found biological filter in veins of the resected sigmoid colon adenocarcinoma tissues (**Figure 5A**) [15], Malignant cancer cells were found in the biological filter, which interfered with hematogenous metastasis of cancer cells (**Figure 5B**).

We previously reported virus-like microorganisms in the T lymphocytes of peripheral blood of pulmonary artery hypertension and VTE patients with low CD3 and CD8 [16] and rod-like bacteria in the phagocytes of peripheral blood in patients with recurrent PE [17]. The heterophilic antigens (pathogenic microorganism or malignant cells) cannot be timely or effectively cleared, indicating that the human body needed to build a new defense line when losing functions of immune cells.

*Inevitability of building a new intravenous defense line*

tor Xa was distributed on the mesh-like structure which was composed of fibrin/fibrinogen.

People invented different types of artificial vena caval filter used in clinics, the mechanism of which is preventing the genesis of PE by blocking the deep venous thrombi flowing back to pulmonary arteries through the filter (**Figure 4B**, Left). Core proteins of thrombi, integrin β2 and β3, bind their ligand fibrinogen to construct mesh structure, which becomes a nest-like filter in thrombi (**Figure 4C**, right).

As a precise and perfect life entity, the regulation of human body is always towards to the beneficial aspects and the balance, stability and extension of internal and external environments. The construction of intravenous biological filter is the result of self-regulation of human body. But what are the effects of biological filter on human body? And what are the meanings of

The defense system existed inside human body is the immune system. Simply speaking, it is the function of immune system that removing all the foreign agents inside human body, including external pathogenic microorganism, implants, invasive foreign bodies and toxin from wounds; and internal generated senile cells and malignant cells. Through a long-term evolution, the immune system with both inside and outside functions has developed perfect tissue structures and exceptional functions. The immune system can be divided into innate immune system and adaptive immune system. The innate immunity, also called congenital immunity, was the oldest existing functionality in the biological evolution, which eliminates the encountered foreign bodies immediately through its components, macrophages, granulocytes, natural killer cells and complement system. However, the adaptive immunity is acquired after birth with a characteristic of hav-
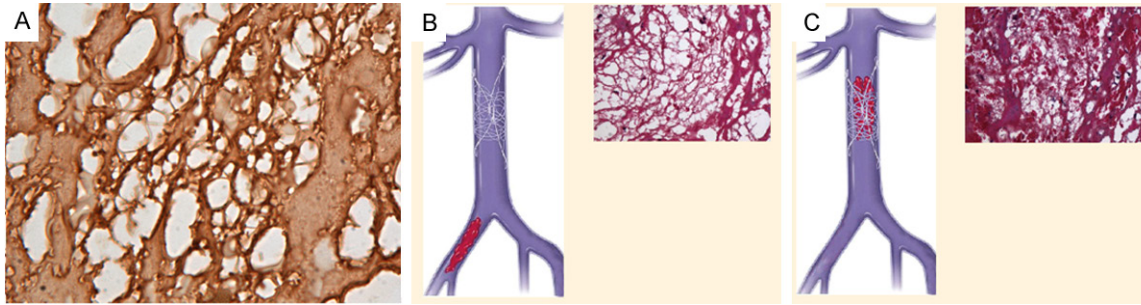
**Figure 4.** A. Immunohistochemistry showed dark-brown integrin β3 was expressed on the platelets which aggregate the skeleton of thrombus and bind with fibrinogen to generate a mesh-like structure (anti-fibrinogen antibody, 1:100, ×1000). B. (Left) Mode pattern of artificial nest-like inferior vena caval filter. (Right) Mesh-like structure is nest-like biological filter in thrombi under microscope. C. (Left) Mode pattern of artificial nest-like inferior vena caval filter filled with thrombi. (Right) Mesh-like structure was nest-like biological filter, in which red blood cell dominant blood cells filled under microscope.
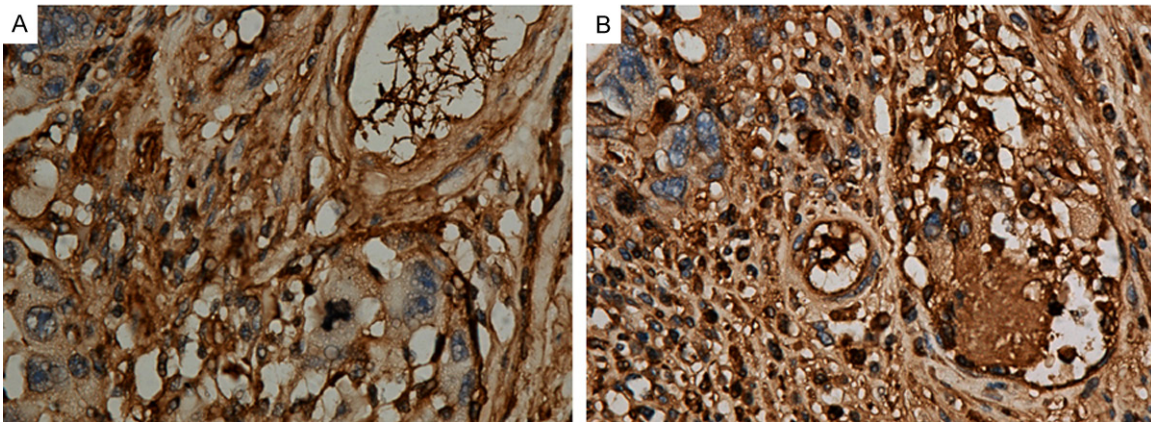


**Figure 5.** A. In colon cancer, massive mesh-like structure (anti-fibrinogen antibody, 1:00, ×400) was observed in venules, and cancer cells were also observed in this mesh-like structure (anti-fibrinogen antibody, 1:100, ×400). Mesh-like structure was nest-like biological filter. B. Pathological findings of cancer tissues showed Intravenous biological filter was filled with cancer cells.

ing memories, which can specifically attack the same invasive foreign agents. That's why the adaptive immunity is also named specific immunity, which functions mainly through T and B lymphocytes. Among them, T lymphocyte is the key of all the immune cells.

The process of immune balance is a complicated process. When the functions of immune cells collapse, the human body will lose the defensive functions of immune system, the alloantigens intrude into the circulating system, and then the alternative defensive barrier is activated inevitably. It is a basic principle of releasing compensatory reserved functions. The formation of intravenous biological filter indicates the existence of alloantigenic cells in the veins and the supplement of the lost immune functions.

*Significant down-regulation of human immune system related gene mRNA expression*

Human genomics is characterized with wholeness, comprehensiveness and directivity. Although there is difference in the gene-guided protein synthesis among individual proteins which requires to be validated by proteomic and cytological studies, comparisons of gene expression patterns among different groups and functional analysis of differentially expressed genes may provide a general view and a direction for the understanding of mechanisms underlying the pathogenesis of diseases. This is a unique feature of genomics, which is not owned by other methods.

1) Gene Ontology analysis of the gene expression in PE group targeted the significant down

regulations of T cell immune complexes and T cell immune functions when compared with the controls [18].

2) Innate immunity: phagocytes, NK cells, complement system and cytokine related gene expressions in both PE group and control group were compared.

(1) Phagocytes: mRNA expressions of pattern recognition receptors (TLR2, TLR4, CD14, MYD88, MRC1L1, MRC2, MSR1, SCARA5, SCARB2, SCARF1 and SCARF2) and opsonic receptors (CR1, FCGR2A, FCGR2B, FCGR3A and FCGR3B) were up-regulated in phagocytes of the PE group compared with controls, among which TLR4, CD14, MYD88, SCARB2, SCARF2, CR1 and FCGR2A were significantly up-regulated (P<0.001), indicating the increased functions of neutrophils and monocytes [19].

(2) NK cells: Compared with control group, NK cells related gene expressions declined overall in PE group, among which mRNA expressions of seven tenth of the lectin-like receptors and Natural cytotoxic receptors were significantly down-regulated (P<0.05), suggesting the reduced the functions of NK cells killing target cells directly [19].

(3) Complement system: There are 14 genes of complement early components. In PBMCs from PE patients, expression of the genes encoding C1qα, C1qβ, C4b and Factor P was significantly greater (P<0.01) than that in controls. Gene expression of MBL and MASP1 was lower (P<0.05) in PBMCs from PE patients compared with controls. Seven genes of the complement late components were detected. In PE patients, mRNA expression of C5 was significantly up-regulated (P<0.05), whereas C6, C7 and C9 were significantly down-regulated (P<0.05) compared with controls. In PE patients, expressions of all the seven genes mRNAs were up-regulated, and mRNA expressions of CR1, integrin αM, integrin αX and C5aR were significantly up-regulated (P b 0·01) compared with controls. Gene expressions of complement regulators C4b binding protein, α (C4BPα), C4b binding protein, β (C4BPβ), Factor H, Factor I, CD59, CD55 and CD46 in PBMCs from PE patients and controls were detected CD59 and CD55 mRNAs were both significantly up-regulated (P<0.05), while Factor I mRNA was significantly down-regulated (P<0.05) in PBMCs from PE

patients than controls, and the other three gene showed down-regulated trend. mRNA expression of various components, receptors and regulators of the complement pathways were unbalanced in PE patients, indicating that the interruption of complement system cascade reactions and the loss of complement mediated membrane attacking functions [20].

(4) Cytokines: In PBMCs from PE patients, the expression levels of genes encoding IFNα5, IFNα6, IFNα8, IFNα14, IFNκ, IFNω1 and IFNε1 were significantly lower than those detected in PBMCs from controls (P<0.05). IFNγ mRNA expression was significantly downregulated in PBMCs from PE patients compared with controls (P<0.01) [21].

A total of 37 interleukin genes were detected. In comparison with the control, the expression levels of 12 genes were downregulated specifically IL1A, IL9, IL17B, IL19, IL23A and IL25 (P<0.05), IL2, IL3, IL13, IL22, IL24 and IL31 (P<0.01), and those of two of the genes were upregulated, specifically IL10 and IL28A (P<0.05), in the PE patients. The imbalance of Th1/Th2 manifests as reduced cell-mediated immunity [22].

Chemokines: Twelve genes encoding CXC chemokines were detected. In PE patients, mRNA expression levels of Cxcl1, Cxcl2, Cxcl6, Cxcl13 and Cxcl14 were significantly upregulated (P<0.05), and Cxcl10 mRNA expression levels were significantly downregulated compared with controls (P<0.01). Twenty-three genes encoding CC chemokines were examined and the mRNA expression levels of CC chemokines were significantly lower in PE patients than controls (P<0.01) [21].

TNF: Thirty-eight genes encoding members of the TNF superfamily and TNF receptor superfamily were examined. In PE patients, the mRNA expression levels of TNF superfamily members 1, nine and 13, and TNF receptor superfamily members 1A, 1B, 9, 10B, 10C, 10D and 19L, were significantly upregulated (P<0.05), whereas TNF receptor superfamily members 11B, 19 and 25, were significantly downregulated compared with controls (P<0.05) [21].

Colony stimulating factor: Six genes encoding colony stimulating factors were detected and the mRNA expression levels of granulocyte-macrophage colony stimulating factor (GM-

CSF), granulocyte colony-stimulating factor (G-CSF), erythropoietin (EPO), thrombopoietin (THPO) and mast cell growth factor (KITLG) were significantly lower in PBMCs from PE patients than controls (P<0.05) [21].

Other cytokine: Eight genes associated with transforming growth factor (TGF), epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF) were detected. The mRNA expression levels of TGFβ1, TGF β1-induced transcript 1, EGF and VEGF were significantly upregulated (P<0.01), whereas TGF β3 mRNA was significantly downregulated (P<0.05) in PBMCs from PE patients compared with controls [21].

From the characteristics of a variety of cytokine mRNA expression levels in PE patients, we conclude that the immune function and the ability of clearing viruses, intracellular bacteria and parasites are reduced in PE patients [21].

(5) In patients with PE, the expression of the majority of integrin mRNAs located on leukocytes and platelets was significantly upregulated. The expression of mRNAs related to L-selectin and P-selectin glycoprotein ligand was significantly upregulated, while the expression of mRNA related to E-selectin was significantly downregulated. The expression of mRNAs related to classic cadherins and protocadherins was downregulated, and the expression of mRNAs related to vascular endothelial cadherin was significantly downregulated; the expression of mRNAs related to the immunoglobulin superfamily had no obvious difference between the two groups. In conclusion, we demonstrated that, in symptomatic PE patients, the adhesion of leukocytes and platelets was enhanced; the activation of endothelial cells was obviously weakened; the adherens junctions among endothelial cells were weakened, with the endothelium becoming more permeable [23].

(6) Among the 13 leukocyte-related integrin mRNAs, integrins β1 and β2 mRNAs expressions were upregulated in the PE group, compared with the controls (P<005). Of the seven platelet-related integrin mRNAs, integrins β2 and β3 mRNAs expressions were upregulated in the PE group, compared with the controls (P<0.05). Among the 11 other integrin mRNAs,

six were upregulated (of which three significantly) in the PE group (P<0.05) and five were downregulated (of which three significantly) (P<0.01). It can be concluded that most leukocyte- and platelet- related integrin mRNAs were upregulated in the PE group, as well as fibronectin- and fibrinogen- related integrin mRNAs [23].

3) Adaptive immunity: T and B lymphocyte related gene expressions in both PE group and control group were compared.

(1) T lymphocyte: Of the six genes of T cell immunological synapse, receptor complex, plasma lemma and receptors mRNAs, ZAP70, CD247 and GZMB mRNAs were downregulated in the PE group, compared with the controls (P<0.05), while GZMA, CD3G and CD3D mRNAs downregulated in the PE group, compared with the controls (P<0.01) [19].

(2) B lymphocyte: mRNA expressions of 82 genes involved in B cell activation were detected. 1) B cell receptor: In PE patients, expressions of LYN, CD22, SYK, BTK, PTPRC and NFAM1 were significantly higher, whereas expressions of FYN, FCRL4 and LAX1 were significantly lower than the control group. 2) T cell dependent B cell activation: In PE patients, mRNA expressions of EMR2, TNFSF9, CD86, ICOSLG, CD37 and CD97 were significantly upregulated, whereas SPN mRNA was significantly down-regulated compared with the control group. 3) T cell independent B cell activation: LILRA1 and TLR9 mRNAs were significantly upregulated in PE patients compared with the control group. 4) Regulators: In PE patients, expressions of the genes including CR1, LILRB4 and VAV1 were significantly higher, whereas expression of SLAMF7 was significantly lower than those in the control group. 5) Cytokines: In PE patients, expressions of genes including LTA and IL10 were significantly higher, whereas expressions of L1A, IFNA5, IFNA6, IFNA8, IFNA14, IL2, IL13 and IFNG were significantly lower than those in the control group. It is indicated that Deferential gene expressions in different stages of B cell activation suggest the decrease or disorders of B cell function [24].

The whole result of the genomics showed that significantly decreased functions of T lymphocytes, disorganized functions of B lymphocytes and complements, and inflammations with enhanced immune adherence.
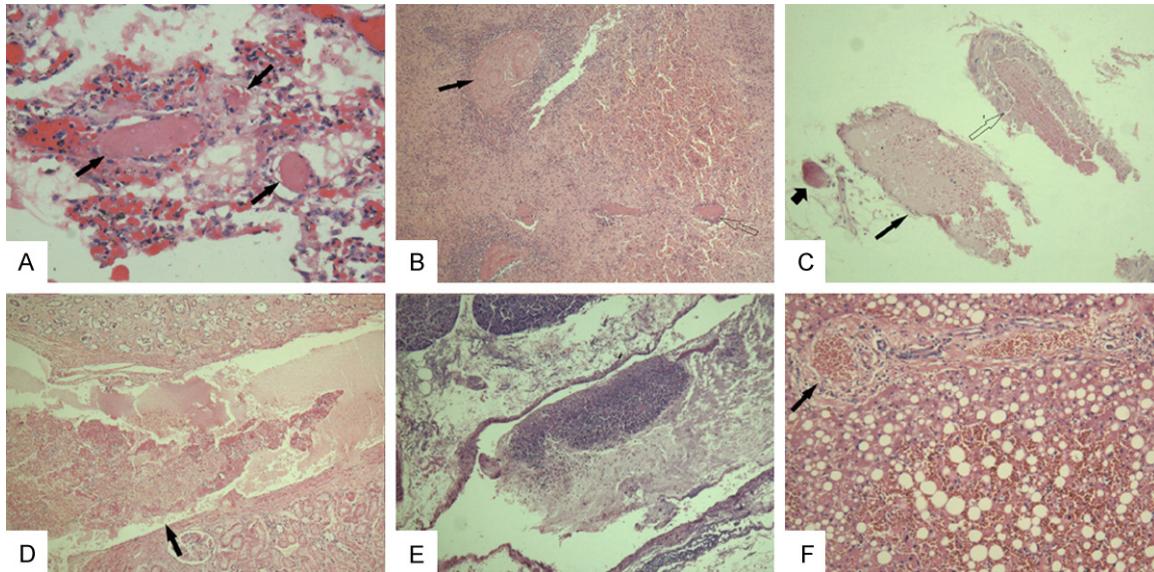
**Figure 6.** A. Lung: thrombi in the lumen of the small vessels (arrow) and significant pulmonary edema and hemorrhage. HE×200; B. Spleen: thickening and hyaline degeneration of the splenic central artery (solid arrow); and the hyaline thrombi in small vessels (open arrow). HE×40; C. Lymphatic system: thrombosis within small vessels in the soft tissue surrounding the mesenteric lymph nodes: hyaline thrombus (thick arrow), mixed thrombus (thin arrow) and red thrombus (open arrow). HE×100; D. Renal interstitium: mixed thrombi (arrow) composed of fibrin, platelets and red blood cells. HE×40; E. Pancreatic lobular interstitium: aggregation of white blood cells and fibrin clot, incomplete occlusion in the vascular cavity (arrow) and perivascular interstitial edema. HE×40; F. Liver: Vascular dilatation and congestion in the peri-portal region are shown (arrow). HE×100.
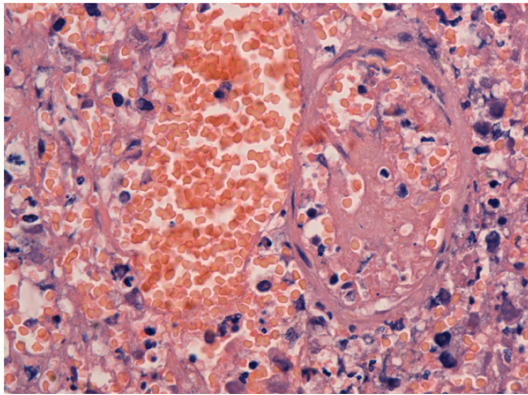


**Figure 7.** Necrotic region in poorly differentiated gastric carcinoma presented with exudation of a large number of red blood cells.

Smeeth et al. [25] reported that the risk for DVT was increased by 1.91 folds within two weeks to six months after acute infection. In two large case-control studies [26, 27], results also demonstrate that acute infection increases the risk for VTE by 2~3 folds after adjustment of other risk factors of VTE. The author's team found DVTs in multiple organs like pulmonary arteries, kidney, liver and pancreas during autopsy of SARS patients [28], indicating the genesis of VTE was related to virus infection (**Figure 6**).

In acute PE [29], the decreasedCD3 and CD8 levels, and the increased CD4/CD8 ratio, were similar to those in CTEPH [30]. We have reported that the function of CD3, CD8, CD16+56 and CD19 was compromised or disordered in more than 95% acute symptomatic VTE [31].

Malignancy is one of risk factors of VTE, and also one of the leading causes of death in patients with malignancy [32-34]. The prevalence of VTE in patients with malignancy is 4-7 times higher than that of patients without malignancy [35, 36].

Malignancy results from that cancer cells cannot be effectively and timely cleared by the immune system. The author [15] reported necrosis, granulation tissues, disruption of small veins, and dark brown fibrinogens in veins formed mesh-like structure in sigmoid colon adenocarcinoma (**Figure 5**). Also, necrotic region in poorly differentiated gastric carcinoma presented with exudation of a large number of red blood cells (**Figure 7**). VTE and hemorrhage serve as inevitable products in the proliferation of cancer cells and disruption of surrounded veins and arteries.

T cell is the key of immune cells, which regulate the cellular immunity, humoral immunity, and

phagocyte functions in innate immunity through Th1, Th2 and Th17. The immune functions decrease, when T cell functions decline. The process of immune balance is a complicated process. Different immune functioning status, including normal, decreased and disturbed immune functioning status, decides different body status. The collapse of the balancing functions of immune cells indicates that immune cells in the innate and adaptive system have a state of no function or dysfunction. The author confirmed significantly decreased T cell functions through cytology, and decreased whole immune cell functions through genomics, indicating the collapse of the balancing functions of immune cells in VTE.

*Expression of same/different proteins in venous thromboembolism and different risk factor group patients*

In our study [35, 36], a total of 1006 subjects were recruited and divided into VTE group, risk factor groups and control (non-risk factor) group without any difference in age. Flow cytometry was performed to detect the expression of the core proteins in venous thrombi. The normal range of integrin β1, β2 and β3 were generated from healthy people.

Compared with control group, the integrin β1 expression in VTE group and subjects with different risk factors (acute infection, malignancy and autoimmune diseases) increased markedly. However, compared with control group, the integrin β1 expression in trauma/surgery group was not significantly different. The elevated expression of integrin β1 in VTE group, acute infection group, malignancy group and autoimmune diseases group was highly consistent with the increased core protein expression in thrombi, indicating that VTE patients shared the same protein expression with these patients with acute infection, malignancy and autoimmune diseases. However, no same elevation of protein expression was found in patients with trauma/surgery.

Compared with control group, the integrin β2 expression in VTE group increased significantly. However, compared with control group, the integrin β2 expression in subjects with different risk factors (acute infection, malignancy and autoimmune diseases, trauma/surgery group) was not significantly different, suggesting no

same elevation of protein expression was found in patients with acute infection group, malignancy group and autoimmune diseases group, trauma/surgery group.

Compared with control group, the integrin β3 expression in VTE group was elevated. However, the integrin β3 expression in different risk factor groups (acute infection, malignancy, autoimmune diseases, trauma/surgery) were not significantly different. It is indicated that integrin β2, β3 are the protein to distinguish patient with VTE from patient with risk factors, and are also the key proteins of determining the occurrence of VTE. The increased integrin β1 is the characterized expression in patients with risk factors. However this increased expression of integrin β1, β2, β3 was not found in patients following trauma or surgery calling into question that such patients may have no VTE risk.

*Core proteins may serve as new specific protein markers of VTE diagnosis*

The author [37] adopted ROC curve analysis to assess diagnostic performance of these core proteins in 120 VTE patients. When a comparison was made between VTE patients and non-VTE patients plus healthy controls, the AUC of integrin β1, integrin β2 and integrin β3 was 0.870, 0.821 and 0.731, respectively. Optimum cutoffs of integrin β1, integrin β2 and integrin β3 calculated according to Youden's index were 10.29 pg/ml, 91.10 pg/ml and 10.35 pg/ml, respectively. With these optimum cutoffs, the sensitivity, specificity, positive predictive value and negative predictive value of integrin β1 were 80.3%, 83.7%, 71.1% and 89.3%, respectively; integrin β2 78.6%, 73.7%, 59.4% and 87.6%; integrin β3 68.4%, 71.2%, 54.3% and 81.8%. The AUC of combined three integrins was 0·916, the sensitivity, specificity, positive predictive value and negative predictive value were 84.6%, 90.8%, 81.7% and 92.0%, respectively. Clinical researches have confirmed significantly increased expression of integrin β1, integrin β2 and integrin β3 in VTE patients, which had relatively high specificity and sensitivity.

Taken together, the significantly decreased immune cell function or the collapse of immune cell balancing function is the upstream condition of acute venous thromboembolism. Cells infected by foreign pathogenic microorganism

and malignant cells trigger the intravenous immune adhesive inflammation, which is the defensive reaction in human body to establish the intravenous biological filter preventing infected cells and malignant cells from flowing back. However, the red thrombus forms and the defense transfers into thrombotic disease, when the filter is filled with red blood cell dominant blood cells.

People with collapsed immune cell balancing functions are the certain groups of venous thromboembolism. Anyone who had venous thromboembolism indicates that there are infected cells and malignant cells in the veins, and which trigger the genesis of venous thromboembolism. Only under the condition of immune cell balancing function collapse, the risk factors, such as advanced age, infection, malignancy, autoimmune disease, pregnancy, long trip syndrome, as well as family history may cause venous thromboembolism. However, even with definable risk factors, there is no risk of getting venous thromboembolism in patients without collapsed immune cell balancing function.

Core proteins in acute venous thrombus are integrin β1, integrin β2 and integrin β3. The increased integrin β1 is the characterized expression in people with risk factors of VTE. However this increased expression of integrin β1, β2, β3 was not found in patients following trauma or surgery calling into question that such patients may have no VTE risk. Trauma or surgery may be not the "true" risk factor for VTE.

It should be extended to the upstream of the disease from the middle and lower reaches to prevent the VTE, to decrease the incidence and to increase the cure rate. Hence, it is not enough only to prevent and reduce the known risk factors, adjustment and improvement of immune cell balancing function, lowering the stress level inside human body, and restoring the balance of neuroendocrine functions, are new contents of prevention, treatment and rehabilitation of VTE.

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## Disclosure of conflict of interest

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