



Venous Thrombus

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1.1 Queries Raised in Clinics

Venous thromboembolism (VTE) includes pulmonary thromboembolism (PE) and deep venous thrombosis (DVT). Both belonging to thrombus, acute arterial thrombus is white thrombus, while acute venous thrombus is red thrombus. What does the pathological difference mean? Venous thrombosis can autolyze, while arterial thrombosis cannot. For VTE patients, oral anticoagulants are usually recommended for 3, 6, or 12 months and occasionally lifelong, but the course cannot be determined. Furthermore, even with standard anticoagulation therapy and INR, some patients still develop chronic thromboembolic pulmonary hypertension (CTEPH). Thus, the physicians are extremely puzzled about anticoagulant usage. Proposed risk factors for VTE include advanced age, infection, malignancy, autoimmune disease, surgery, trauma, pregnancy, long trip syndrome, family history, AMI, heart failure, and so on. Relevant risk factors are increasing over time. Risk factors are derived from the summary of evidence-based medicine. Although these factors are found to be associated with venous thrombosis, the intrinsic factors have not been well elucidated [1–4].

1.2 VTE, a Disease Faced by Clinicians in Different Departments

Clinically diagnosed VTE is also known as dominant VTE, but the diagnosis of insidious VTE is very difficult, and it is often found at autopsy. DVT of cerebral cortical vein and cerebral venous sinus, acute PE, chronic thromboembolic

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pulmonary hypertension, hepatic venule occlusion, DVT of mesenteric vein and pelvic vein, postoperative DVT and PE, and lower limb DVT are diagnosed in different departments, and thus VTE is almost a disease faced by clinicians in all departments. Of these DVTs, PE has a high morbidity, high misdiagnosis rate and high mortality and has been an important health problem worldwide [1, 2]. VTE can be classified as acquired and hereditary VTE. Epidemiological studies reveal that hereditary VTE has a low morbidity, and VTE is largely acquired VTE. Both hereditary VTE and acquired VTE can be grouped into symptomatic VTE if one can't be differentiated from the other (Figs. 1.1, 1.2, and 1.3).

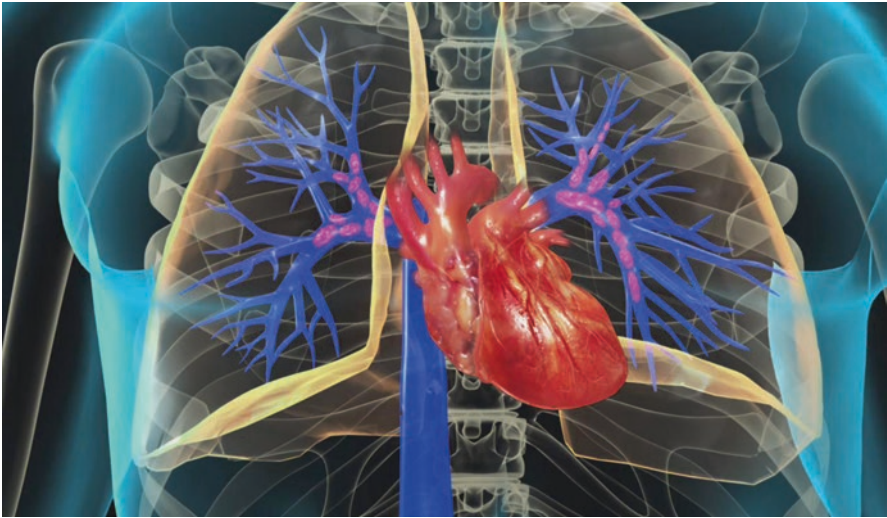


Fig. 1.1 Diagram of pulmonary thromboembolism

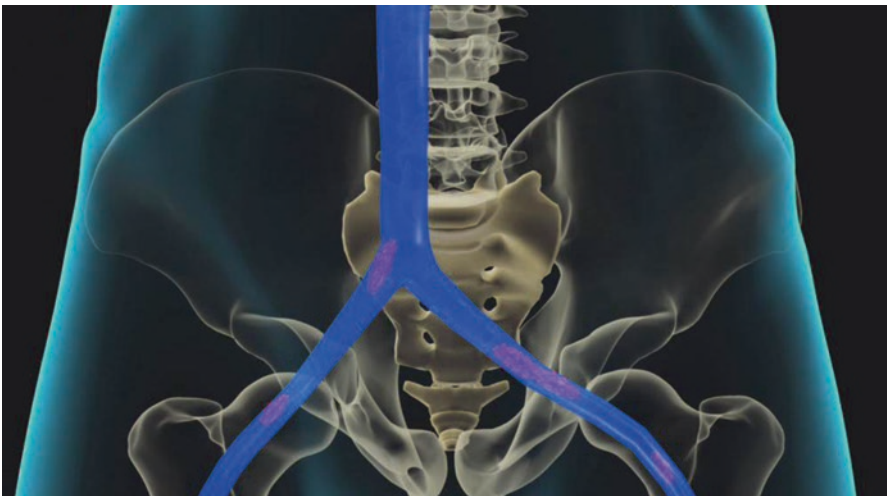


Fig. 1.2 Diagram of iliac vein thrombosis



Fig. 1.3 Diagram of lower extremity deep venous thrombosis

1.3 Protein Analysis of Acute Venous Thrombosis

Several thrombi were extracted from the pulmonary artery of PE patients. In acute venous thrombosis, thrombus is microscopically red and is composed of red blood cells, platelets, white blood cells, and plasma proteins under a microscope. The thrombus in acute venous thrombosis is often fragile (Fig. 1.4).

Mass spectrographic analyses showed that the majority of proteins in the acute venous thrombus were fibrinogen; the remaining proteins included serum albumin and cytoskeletal proteins (Fig. 1.5). The reversible combinations between fibrinogen and their ligands theologially explain that acute venous thrombus is easy to autolyze; delayed thrombolysis is effective, and the thrombus is easy to lyse through interventional fragmentation [5].

In acute venous thrombosis, the thrombus is red and is composed of red blood cells, platelets, white blood cells, and fibrinogen. How does fibrinogen bind to blood cells in the thrombosis? MS/MS and bioinformatics analysis of the thrombus from acute PE patients showed that integrin subunits β_1 , β_2 , and β_3 were the core proteins of the thrombus (Figs. 1.6 and 1.7).

Integrins are important members in the cell adhesion molecule family and mediate the adhesion between cells and between cells and extracellular matrix (ECM) as well as the bidirectional signal transduction between cells and ECM. Integrins can bind to some ligands related to cell activities and are involved in some physiological and pathological processes, including angiogenesis, invasion and metastasis of cancers, inflammation, wound healing, and coagulation [6].

Integrin is a transmembrane heterodimer composed of subunits α and β at a ratio of 1:1 (Fig. 1.8). To date, a total of 18 α subunits and 8 β subunits have been identified, and they can form 24 functional heterodimers, which may be classified into 8

Fig. 1.4 HE staining of the venous thrombus. Red thrombus was observed and composed of massive red blood cells and white blood cells with dark-brown nuclei aggregated (HE staining, $\times 200$) (Reprinted with permission from *Int J Clin Exp Med* 2015;8(11):19804–19814 [42])

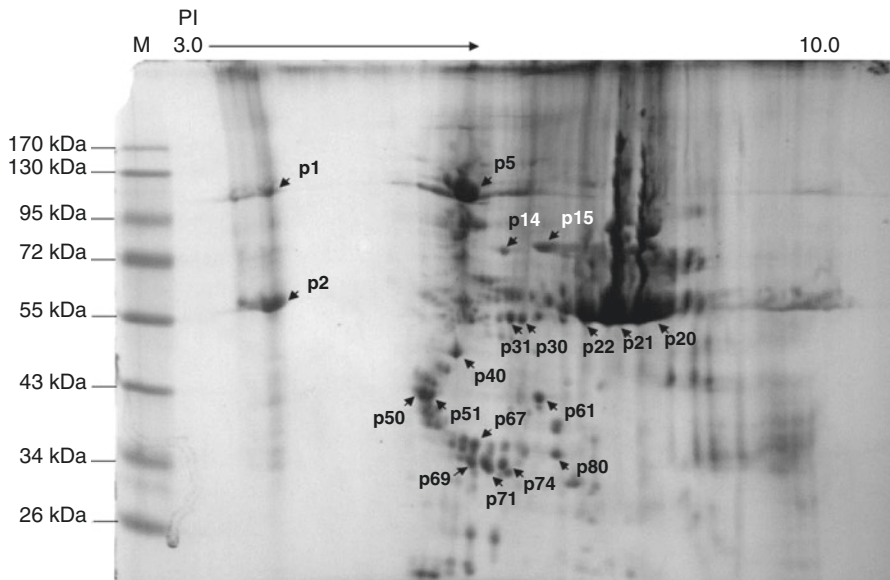
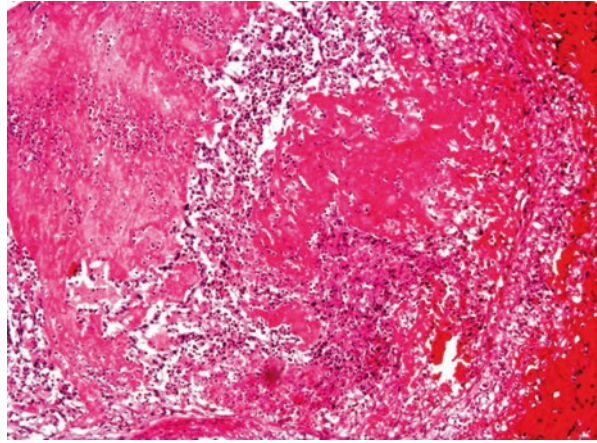


Fig. 1.5 Electrophoresis and mass spectrographic analysis of proteins in the thrombus from an acute PE patient (Reprinted with permission from *Am J Respir Crit Care Med* 2011;184:145–6 [5])

groups (β_1 – β_8) 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 its ligands. 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 (Figs. 1.9 and 1.10), and the β subunit dominates the signal transduction and regulation of integrins affinity [7–9].

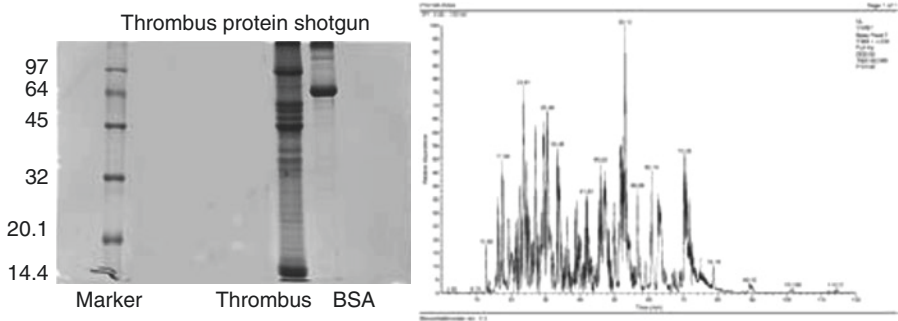


Fig. 1.6 Component analysis of the thrombus. Left, pre-isolation of acute thrombus; right, MS/MS fragment sequence information of acute thrombus

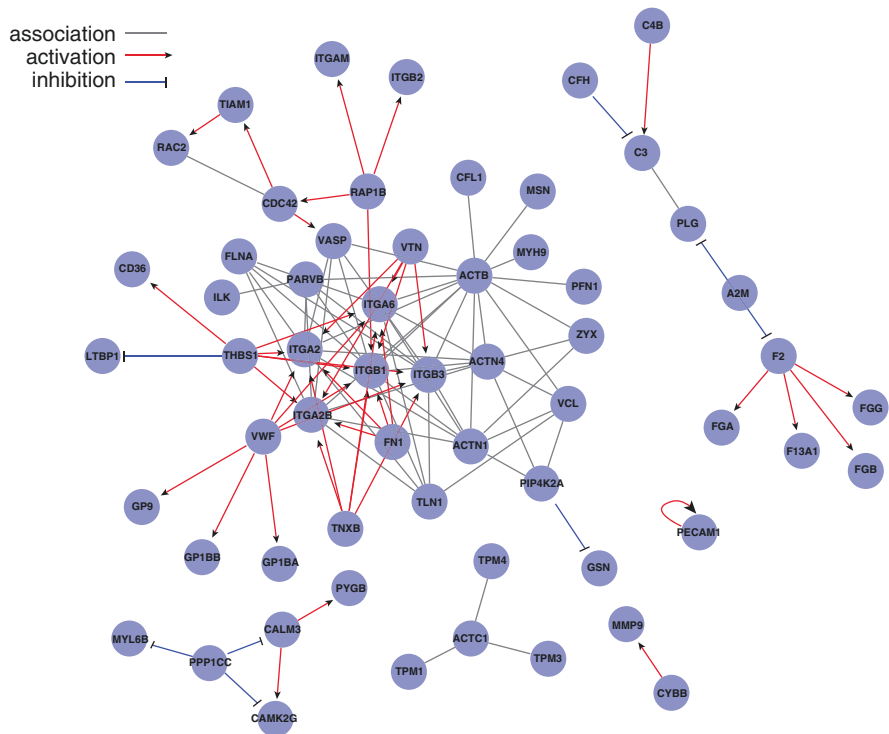


Fig. 1.7 MS/MS and bioinformatics analysis of the thrombus from acute PE patients. Subunits β_1 , β_2 , and β_3 in integrins were the core proteins of the thrombus (Reprinted with permission from Int J Clin Exp Med 2015;8(11):19804–19814 [42])

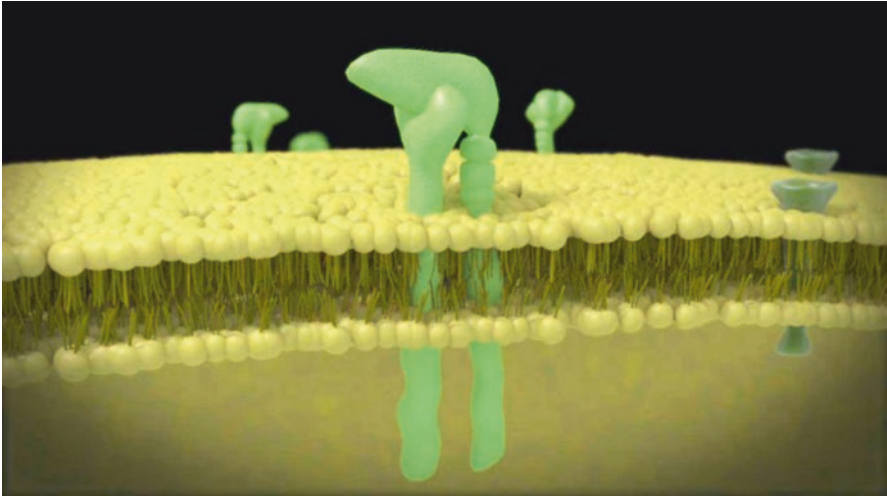


Fig. 1.8 Integrin is a transmembrane heterodimer formed by one α subunit and one β subunit via a non-covalent bond. At resting state, α subunit does not bind to its ligand. Reprinted with permission from *Int J Clin Exp Med* 2015;8(11):19804–19814

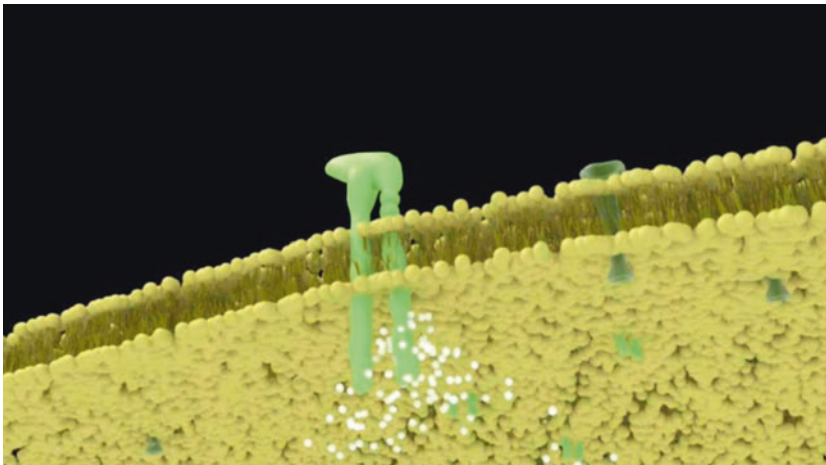


Fig. 1.9 α subunit and β subunit of an integrin are regulated by extracellular signals

1.4 Localization and Expression of Core Proteins in the Acute Venous Thrombosis

The β_1 subunit is mainly found on the lymphocytes and platelets, and its ligands include laminin, collagen, thrombospondin, fibronectin, and VCAM-1. The β_2 subunit is mainly distributed on the neutrophils and monocytes, and its ligands include fibrinogen, ICAM, factor X, and iC3b. The β_3 subunit is mainly observed on the

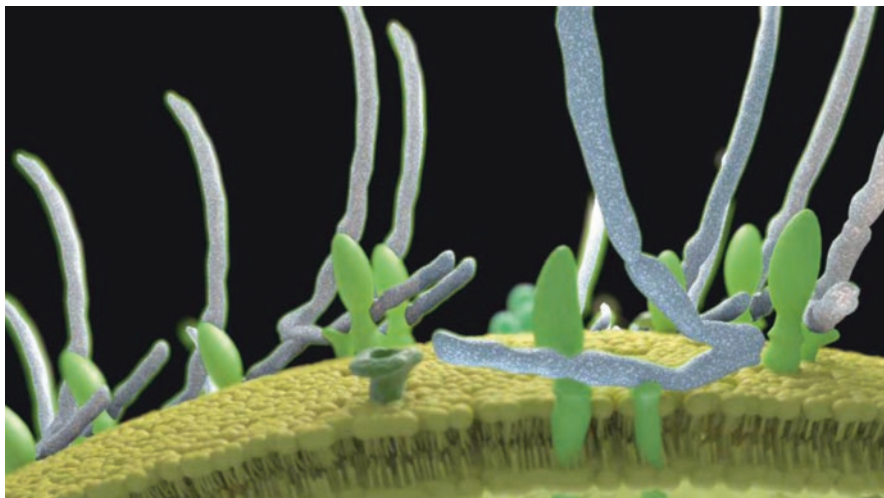


Fig. 1.10 Following integrin activation, α subunit departs from β subunit and binds to its ligand

platelets, and its ligands include fibrinogen, fibronectin, vitronectin, vWF, and thrombospondin [10–12].

The authors reported that a catheter could be used to extract the thrombus from the pulmonary artery of acute PE patients [13]. Immunohistochemistry showed that dark-brown integrin β_1 was expressed on membrane of lymphocytes, but lymphocytes had no expression of laminin, fibronectin, collagen-I, or collagen-II (receptors of integrin β_1) (Fig. 1.11).

Immunohistochemistry showed that dark-brown integrin β_2 was expressed on the membrane of neutrophils, which bound to fibrinogen, whereas ICAM, factor X, and iC3b were expressed on the membrane of neutrophils (Fig. 1.12).

Immunohistochemistry showed that dark-brown integrin β_3 was expressed on platelets, which aggregated to form thrombotic skeleton; these platelets bound to fibrinogen to construct filamentous mesh structure. No expression of fibronectin, vitronectin, or vWF was observed on the platelets (Fig. 1.13).

Dark-brown factor Xa was distributed on the filamentous mesh structure, which was composed of fibrin/fibrinogen (Fig. 1.14).

To prevent PE, different kinds of filters such as vena cava filters were developed 30 years ago and had been applied in clinical practice to block DVT thrombi below the inferior vena cava reflowing to the pulmonary artery by constructing a mesh structure (Fig. 1.15 left and Fig. 1.16 left). Pathological examination of the thrombus showed a biofilter similar to the artificial filter (Fig. 1.15, right), and the biofilter was full of blood cells (Fig. 1.16, right). These findings indicate that biofilter has similar function to artificial filter.

The sophisticated body always adjusts to the favorable direction of development and tends to balance stability and continuity between the internal and external environments. Biological venous filter is a result of the body's own regulation. What is the role of the spontaneous venous biological filter?

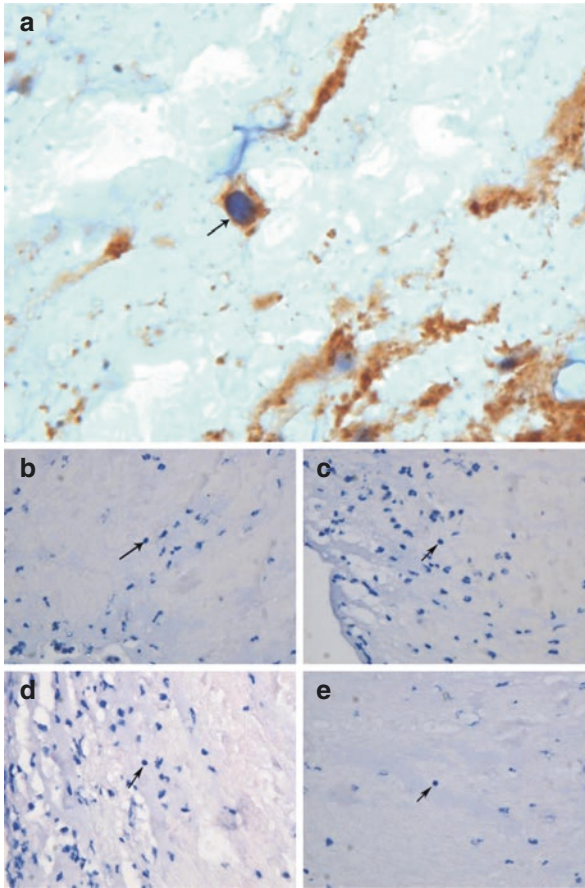


Fig. 1.11 Immunohistochemistry of integrin β_1 and its ligands. Arrow: dark-brown integrin β_1 was expressed on the lymphocytes (**a**, $\times 1000$). Expression of integrin β_1 ligands (laminin, **b**, $\times 400$; fibronectin, **c**, $\times 400$; collagen-I, **d**, $\times 400$; collagen-II, **e**, $\times 400$) was not observed on lymphocytes (Reprinted with permission from *Int J Clin Exp Med* 2014;7(3):566–572 [13])

The central thrombus showed filament-like filter structure (Fig. 1.17a), which was full of erythrocyte-dominant blood cells (Fig. 1.17b). The pathological examination of intestinal cancer showed biofilters in the veins of the cancer (Fig. 1.17c), and cancer cells were found in the biofilters (Fig. 1.17d), suggesting that the biofilter may block the hematogenous metastasis of cancer cells [14].

In acute PE patients (reductions in CD_3^+ T cells and CD_8^+ T cells), electronic microscopy of peripheral venous blood showed virus-like microorganisms in

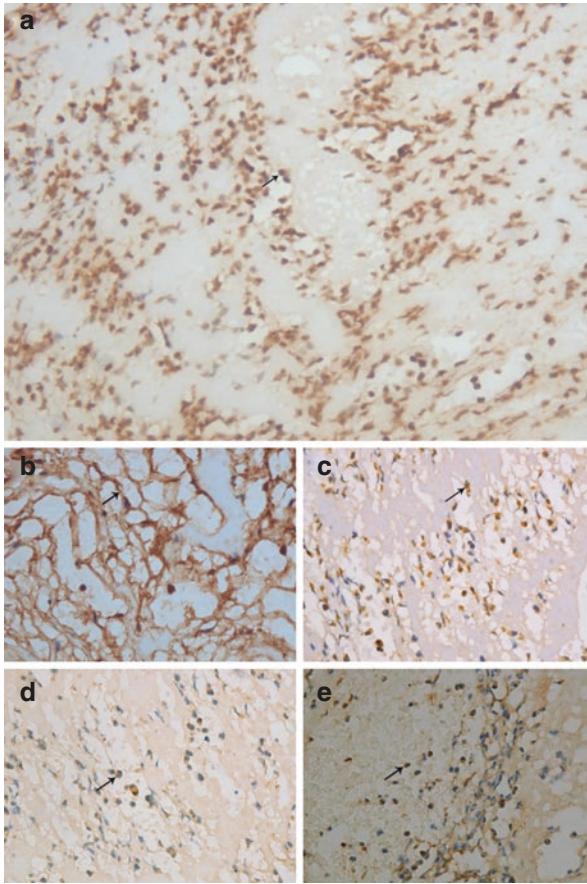


Fig. 1.12 Immunohistochemistry of integrin β_2 and its ligands. Arrow: dark-stained integrin β_2 was expressed on the neutrophils (a, $\times 400$) and bound to fibrinogen (b, $\times 400$). ICAM (c, $\times 400$), factor X (d, $\times 400$), and C3b (e, $\times 400$) were expressed on neutrophils (Reprinted with permission from *Int J Clin Exp Med* 2014;7(3):566–572 [13])

lymphocytes (Figs. 1.18 and 1.19), which seem to be cells with intracellular infection [15].

In patients with repeated PE/DVT (reductions in CD_3^+ T cells and CD_8^+ T cells), electronic microscopy of peripheral venous blood showed rod-shaped bacteria-like structures in the cytoplasm of phagocytes (Fig. 1.20), which seem to be neutrophils with intracellular infection [16].

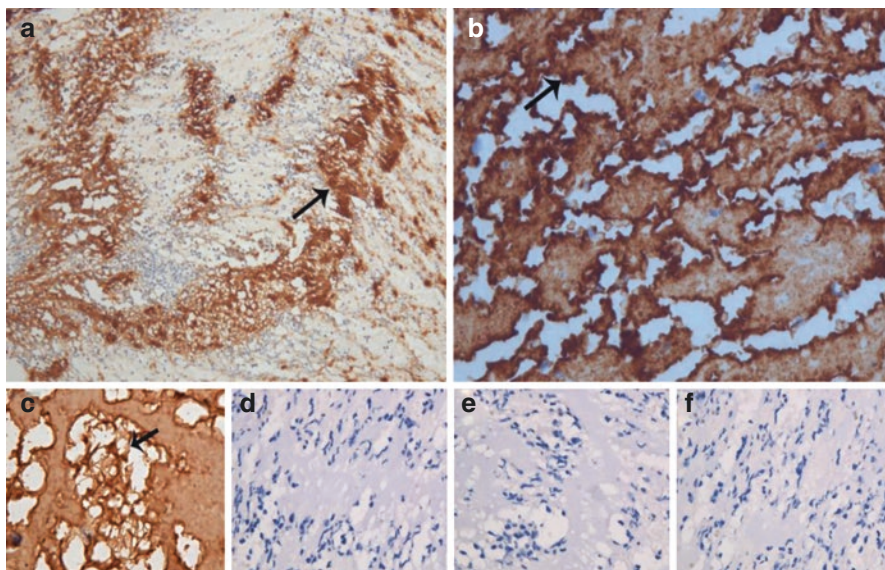


Fig. 1.13 Immunohistochemistry of integrin β_3 and its ligands. Arrow: dark-brown integrin β_3 was expressed on platelets (a, $\times 200$) and on the coral-like skeleton formed by platelets (b, $\times 400$). Platelets and neutrophils bound fibrinogen to construct mesh-like structure (c, $\times 400$). No expression of fibronectin (d, $\times 400$), vitronectin (e, $\times 400$), and vWF (f, $\times 400$) was observed on these cells (Reprinted with permission from *Int J Clin Exp Med* 2014;7(3):566–572 [13])

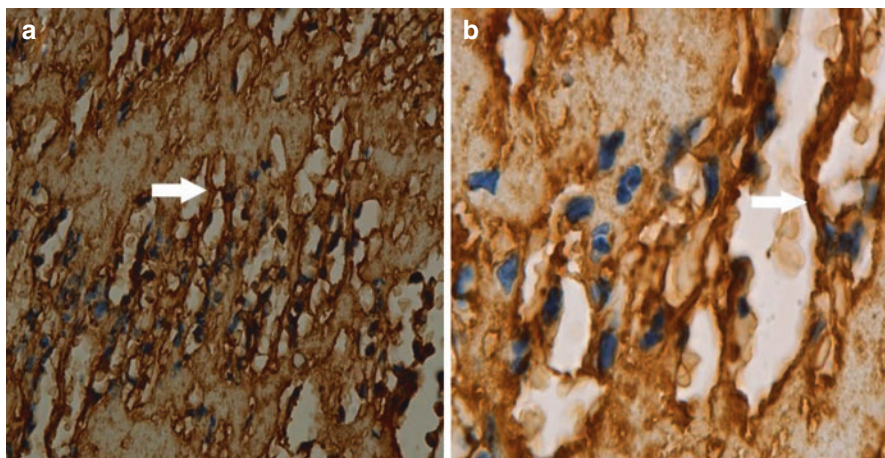


Fig. 1.14 Factor Xa was widely distributed on the surface of mesh-like structure. Arrow: dark-brown factor Xa was found on the surface of mesh-like structure (a, $\times 400$; b, $\times 1000$). This suggests factor Xa acts on the fibrinogen/fibrin (Reprinted with permission from *Int J Clin Exp Med* 2014;7(3):566–572 [13])

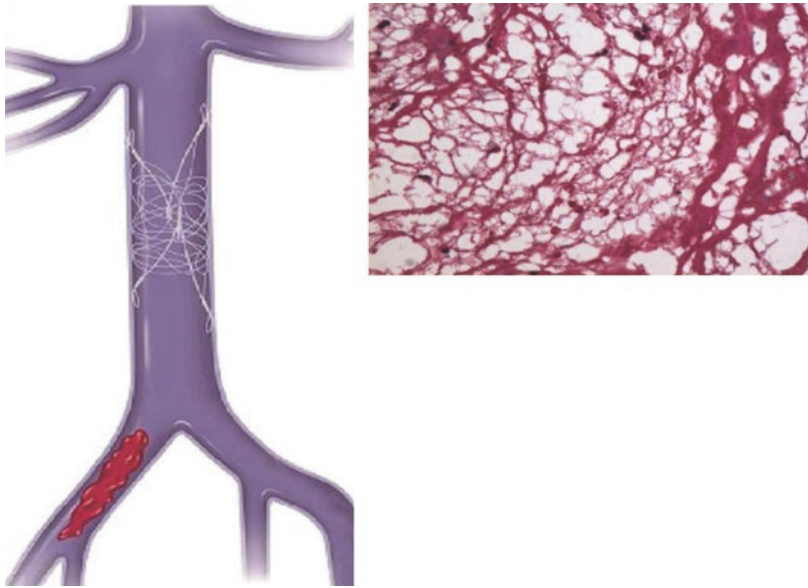


Fig. 1.15 Artificial nest-like inferior vena cava filter and venous biofilter. Left, schema of artificial nest-like inferior vena cava filter; right, nest-like structure of biofilter in the venous thrombus under a microscope (Reprinted with permission from *Int J Clin Exp Med* 2015;8(11):19804–19814 [42])

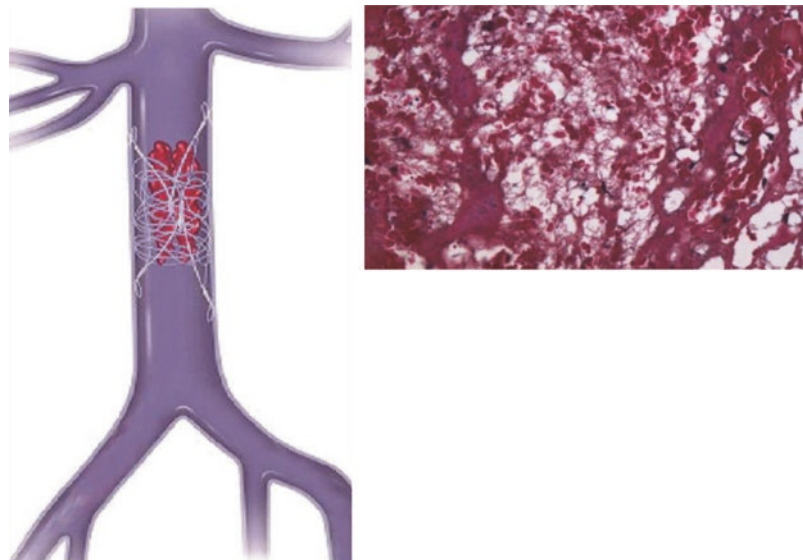


Fig. 1.16 Artificial inferior vena cava filter blocks the backflow of thrombus and blood cells in nest-like structure of the biofilter. Left: schema of artificial nest-like inferior vena cava filter. Backflow of thrombus arrested in the artificial inferior vena cava filter; right, under a microscope, erythrocyte-dominant blood cells were found in the nest-like structure of biofilter in the venous thrombus (Reprinted with permission from *Int J Clin Exp Med* 2015;8(11):19804–19814 [42])

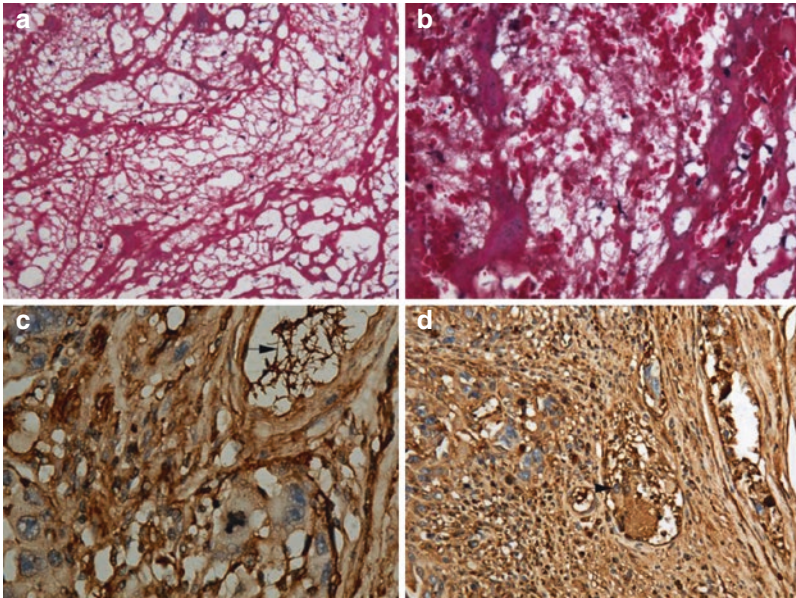


Fig. 1.17 Nest-like biological venous filter is a result of fibrinous inflammation. In acute PE thrombus, there builds a nest-like biological venous filter (**a**, Masson $\times 200$), which was filled with red blood cells (**b**, Masson $\times 200$); biological venous filter formed in veins surrounding cancer tissues (immunohistochemistry for fibrinogen, **c** $\times 400$, arrow); the filter was detained with cancer cells (immunohistochemistry for fibrinogen, **d** $\times 400$, arrow)

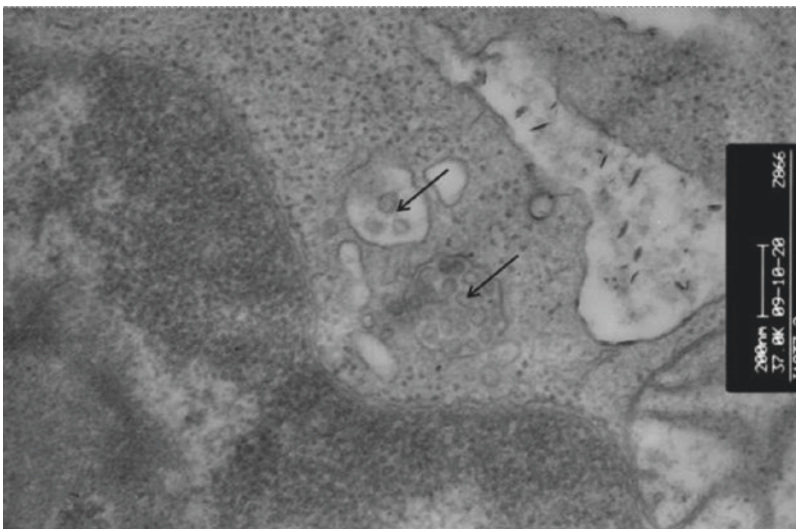


Fig. 1.18 Round structures with intact capsules and different densities of granules in the cytoplasm of T cells (upwards arrow, $\times 37000$). (Reprinted with permission from Am J Respir Crit Care Med. 2010;182(3):434–435 [15])

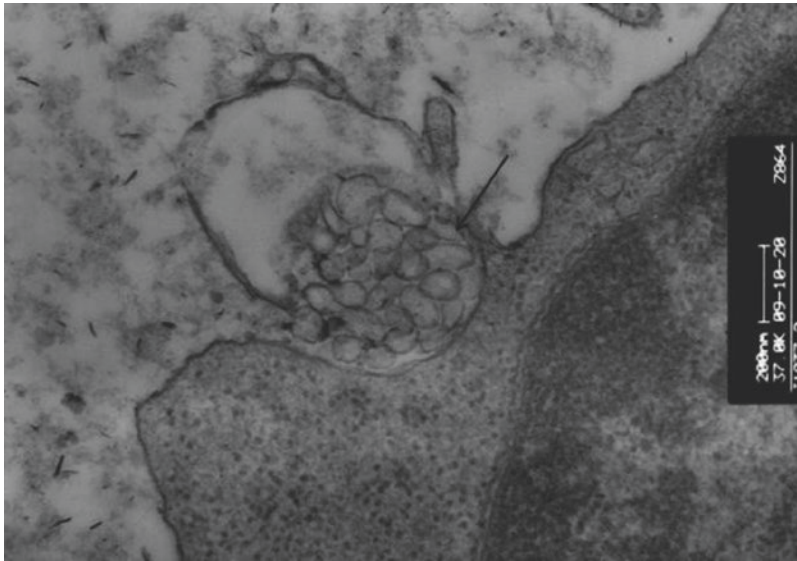


Fig. 1.19 Mulberry-like pathogenic microorganisms penetrated through the T cell membrane and pullulated (upwards arrow, *37000). (Reprinted with permission from Am J Respir Crit Care Med. 2010;182(3):434–435 [15])

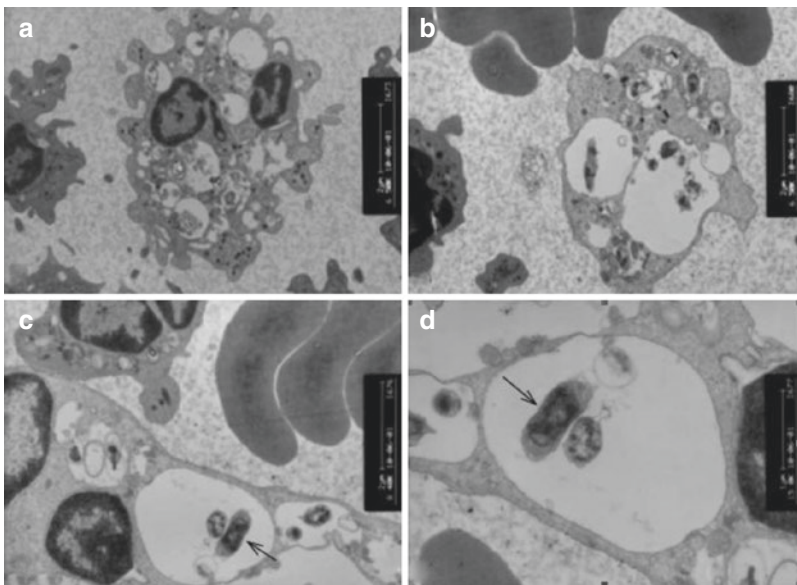


Fig. 1.20 Neutrophil damage and pod-shaped bacteria-like microorganisms under electronic microscope. (a): apoptotic neutrophils; (b) the nucleus of neutrophils disappeared; (c) pod-shaped bacteria-like microorganisms were identified in the cytoplasm of phagocytes(arrow); (d) pod-shaped bacteria-like microorganisms at a higher magnification (arrow) (Reprinted with permission from Am J Respir Crit Care Med. 2012; 186(7): 696 [16])

1.5 Why Biofilter Is Required for the Vein?

The immune system in the human body is the defense system. In simple terms, the immune system functions to expel all the foreign bodies including exogenous pathogenic microorganisms, grafts, foreign bodies in the wound, toxins, senescent cells, and malignant cells. The immune system can be divided into innate and adaptive immunity. Innate immunity is present from birth and is also known as congenital immunity. Innate immune is an evolutionarily ancient defense strategy and is mainly composed of macrophages, granulocytes, NK cells, and complement system. Innate immune can recognize and subsequently clear foreign bodies and then present the information of these foreign bodies to T helper cells in adaptive immune. Adaptive immune is an acquired system during the lifetime of the organism. Immunological memory is a characteristic of adaptive immune and is highly specific to a particular pathogen. Thus, adaptive immune is also known as specific immune system and is composed of T cells and B cells. T helper cells are the core of the immune cells that regulate the cellular immunity and humoral immunity as well as the functions of phagocytes.

Under physiological conditions, the alloantigens (pathogenic microorganisms or malignant cells) in the circulation can be effectively and timely recognized and cleared by immune cells. In the vein, cells with intracellular infection and malignant cells cannot be killed and cleared by immune cells, implying that the immune cells lose their function and are unable to maintain the defense. Under such conditions, a new compensatory defense should be initiated.

The release of reserved compensatory defense is a basic law for life sustainability. The venous defense is manifested by the formation of venous biofilter, which is full of blood cells in the backflow blood, causing venous thrombosis. This may lead to the progression from compensatory defense in the vein into venous thrombosis.

On the other hand, this implies that there are cells with intracellular infection or malignant cells in patients with venous thromboembolic diseases.

1.6 mRNA Profiling of Immune-Related Genes

Infected leukocytes or intravenous malignant cells cannot be timely and effectively killed and cleared, indicating the loss of function of immune cells. This also indicates that the immune system function is changed. Herein, we analyze this phenomenon at the gene and cell levels.

Genomics has the characteristics of wholeness, comprehensiveness, and directivity. Although the gene-guided protein synthesis often displays differences in the synthetic process and protein and cell analyses are usually required to confirm the results, the gene expression profile determined by genomic analysis may reflect the differentially expressed genes, and the functional analysis of these genes may determine global changes in a disease and present a direction to the understanding of the disease. The global analysis of genomics is superior to other tools.

1. Gene ontology analysis showed that the differentially expressed genes in PE patients reflected the downregulation of T cell immune receptor complexes and T cell-mediated immunity as compared to control group [17].
2. In respect of innate immunity, the genes related to phagocytes, NK cells, complements, and cytokines were compared between PE patients and controls.
 - (a) mRNA expression of phagocytes related genes: as compared to controls, the mRNA expression of pattern recognition receptor and conditioning receptors in phagocytes was significantly upregulated in PE patients, suggesting the enhancement of phagocyte function [18].
 - (b) mRNA expression of NK cell-related genes: when compared with control group, the mRNA expression of 7/10 of lectin-like receptors and natural cytotoxic effector receptors reduced significantly ($P < 0.05$), suggesting that the NK cell-mediated killing of target cells was significantly compromised [19].
 - (c) mRNA expression of genes related to complements: when compared with the control group, the mRNA expression of intrinsic ingredients of complements, receptors, and regulatory proteins was disordered, indicating the interrupted cascade reaction of complement system and the loss of complement-mediated membrane lysis [20].
 - (d) mRNA expression of genes related to cytokines: when compared with the control group, the mRNA expression of the following genes changed significantly in PE group.

Interferon gene: mRNA expression of type I interferon reduced ($P < 0.05$), and IFN- γ mRNA expression decreased significantly ($P < 0.01$) in PE group [21].

Interleukin: mRNA expression of Th1-related genes to Th2-related genes reduced in PE group, suggesting reduced killing activity [21, 22].

Chemokines: mRNA expression of 12 CXC-related genes was significantly upregulated ($P < 0.01$) in PE group, and CXCL10 mRNA expression reduced markedly ($P < 0.01$); mRNA expression of 23 CC-related genes reduced dramatically in PE group ($P < 0.01$) [21].

Tumor necrosis factor: 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, 9, 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 to the controls ($P < 0.05$) [20].

Colony stimulating factor: In PE group, the mRNA expression of CSF3, THPO, KITLG ($P < 0.05$), CSF2, and THPO ($P < 0.01$) changed significantly [21].

Other cytokines: In PE group, the mRNA expression of TGFB1, TGFB111, EGF, and VEGF increased markedly ($P < 0.01$), but TGF B3 mRNA expression reduced dramatically ($P < 0.05$) [21].

In PE patients, the mRNA expression of some cytokines was disordered, which reflects reduced killing activity against microorganisms, so the human body is susceptible to infection by virus, intracellular bacteria, and parasite [21].

- (e) In PE group, the mRNA expression of some integrins related to leukocytes and platelets was significantly upregulated; mRNA expression of L-selectin and PSGL-1 increased significantly, but E-selectin mRNA expression reduced markedly; mRNA expression of typical cadherin and cadherin precursor showed reducing tendency, and mRNA expression of VE-cadherin reduced significantly; mRNA expression of immunoglobulin superfamily members remained unchanged. These findings indicate that the leukocyte adhesion is significantly enhanced; the platelet adhesion is markedly enhanced; the activity of endothelial cells is significantly compromised; the adhesion between endothelial cells is markedly compromised, leading to the increased endothelial permeability [23].
- (f) Thirteen differentially expressed genes were leukocyte-related integrin genes. In PE group, mRNA expression of integrin β_1 and β_2 increased significantly ($P < 0.05$); platelet-related integrins ($n = 7$) displayed changed mRNA expression in PE group, of which the mRNA expression of integrin β_1 and β_3 increased significantly ($P < 0.05$); the mRNA expression of other ten integrins was either up- or downregulated. These imply that the mRNA expression of leukocyte- and platelet-related integrins is significantly upregulated, and the mRNA expression of fibronectin- and fibrinogen-related integrins also changed markedly [23].
3. In respect of adaptive immunity, the expression of T cell- and B cell-related genes was compared between the PE and control groups.
- (a) T lymphocyte-related genes in PE group: of six genes related to the T lymphocyte immune synapses, receptor complex, cytoplasmic membrane, and receptors, the mRNA expression of ZAP70, CD247, GZMB ($P < 0.05$), GZMA, CD₃G, and CD₃ D ($P < 0.01$) decreased dramatically [18].
- (b) Genes related to B cells: a total of 82 differentially expressed genes were related to B cell activation. These genes included (1) B cell receptor-related genes (the mRNA expression of LYN, CD22, SYK ($P < 0.05$), BTK, PTPRC, and NFAM1 ($P < 0.01$) was significantly upregulated, but the mRNA expression of FYN ($P < 0.05$), FCRL4, and LAX1 ($P < 0.01$) was markedly downregulated in PE group), (2) T cell-dependent B cell activation-related genes (the mRNA expression of EMR2 ($P < 0.05$), TNFSF9, CD₈6, ICOSLG, CD₃7, and CD97 ($P < 0.05$) was significantly upregulated, but the SPN mRNA expression was markedly downregulated ($P < 0.05$)), and (3) T cell nondependent B cell activation-related genes (the mRNA expression of LILRA1 and TLR9 increased dramatically in PE patients ($P < 0.01$) [24]).
4. Regulator-related genes: In PE group, the mRNA expression of CR1, LILRB4, and VAV1 was significantly upregulated ($P < 0.01$) but that of SLAMF7 reduced markedly ($P < 0.01$) [21].
5. Genes of B cell-related cytokines: In PE group, the mRNA expression of LTA ($P < 0.05$) and IL-10 ($P < 0.01$) increased significantly but that of L1A, IFNA5, IFNA6, IFNA8, IFNA14 ($P < 0.05$), IL2, IL13, and IFNG ($P < 0.01$) reduced markedly. These indicate that there is B cell dysfunction in PE patients [24].

Phagocytes, NK cells, complement system, T cells, and B cells are the important components of innate and adaptive immune systems, and both of them play important roles in the defense against pathogens. These components together exert the defense effects. Results from genomic analysis indicate that the expression of phagocyte-related genes increases significantly but that of NK cell-related genes reduces markedly, the expression of complement-related genes is disordered but that of cytokine-related genes shows imbalance, and the expression of T cell-related genes reduces dramatically and that of B cell-related genes is disordered. These imply that the balance of systemic immune cell function collapses.

1.7 Familial VTE and Characteristics of DNA Sequence Mutations

In a family with familial VTE (Fig. 1.21a), perforin gene mutation was found in three patients [25]. Perforin is localized in NK cells, T cells, and complement. Results showed the combined immune function (killing of target cells) was lost. NK cells, CD₈⁺ T cells, and complement form membrane attack complex to kill the target cells in which the perforin drills a hole in the target cells, leading to the release of granzymes and the death of these target cells. The mutation of the perforin gene may cause the generation of abnormal protein (Fig. 1.21b), resulting in loss of function. In patients with symptomatic PE, genomic analysis showed the mRNA expression of granzyme in T cells reduced significantly, suggesting the compromised killing activity of T cells. Although perforin and granzyme act at different steps of killing of target cells, the gene mutation of perforin in familial VTE patients and reduced mRNA expression of granzyme in patients with symptomatic PE cause the same consequence: compromised killing activity of immune cells [25].

1.8 Immune Function and VTE

Smeeth reported that the pathogenesis of VTE was related to infection, and the risk for VTE increased by 1.91-folds at 2 weeks to 6 months after acute respiratory tract infection [26]. Two large-scale case-control studies also revealed that acute infection could increase the risk for VTE by two- to threefolds after adjusting for other risk factors [27, 28]. In a patient who died of SARS, DVT was found in multiple organs including the pulmonary artery, kidney, liver, and pancreas, suggesting that viral infection is closely related to the pathogenesis of VTE (Fig. 1.22) [29].

A group of patients with acute PE showed significantly compromised function of CD₃⁺ and CD₈⁺ cells [30]; a group of patients with CTEPH displayed markedly compromised function of CD₃⁺ and CD₈⁺ cells [31]; a group of patients with acute symptomatic VTE presented reduced/disordered expression of CD₃⁺, CD₈⁺, CD₁₆⁺, CD₅₆⁺, and CD₁₉⁺, which was observed in 95% of these patients [32]. The results from cytological examination are consistent with those from genomic analysis and both indicate the compromised immune cell balance. T

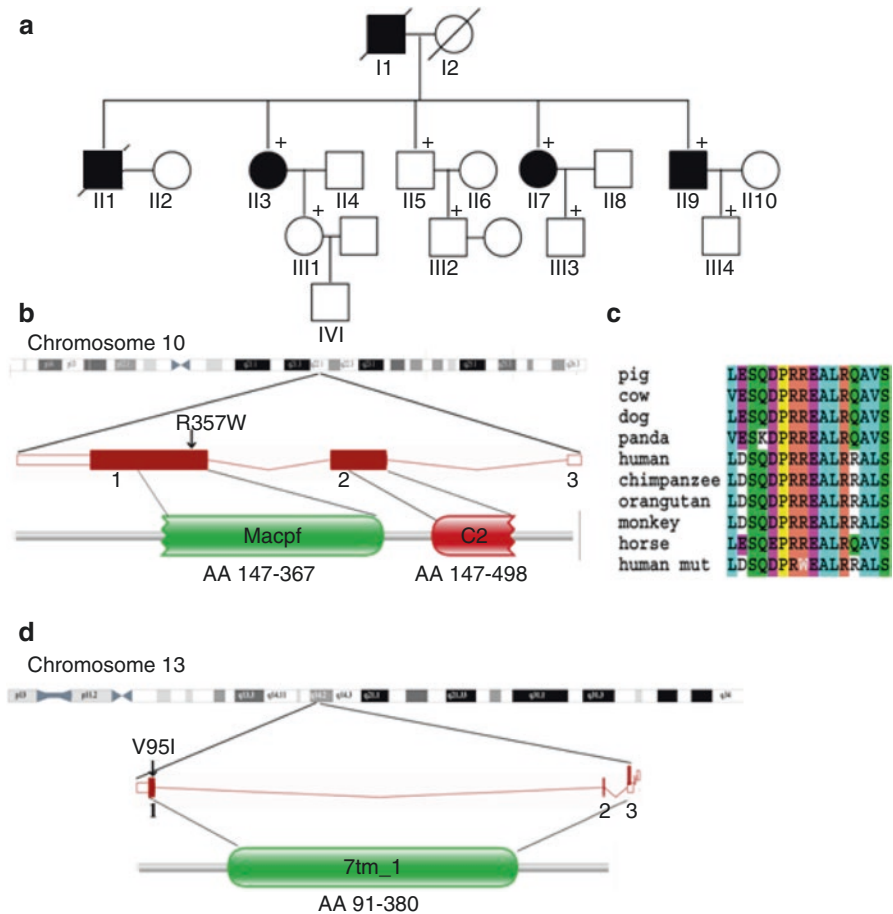


Fig. 1.21 Pedigree and *PRF1* and *HTR2A* mutations of the VTE family. (a) The genealogical tree of VTE family. “+” indicates family members who were examined and sequenced in this study. Solid symbols indicate affected individuals. (b) The chromosomal location and genomic structure of the exons encoding the open reading frame of *PRF1*. The R357W mutation locates in the MACPF domain of protein. (c) *PRF1* orthologous conservation analysis was performed using CLUSTALW at default settings. The *PRF1* R357W mutated sequence is shown in white. (d) The chromosomal location of *HTR2A* and the location of its exons. The exon numbering indicates *HTR2A* is located on the reverse strand, and the V95I mutation is located on exon 1 (Reprinted with permission from Int J Clin Exp Med 2015;8:7951–7 [25])

cells are the core component of immune cells. In T helper (Th) cells, Th1, Th2, and Th17 cells may regulate cellular, humoral, and innate immunity, respectively. The compromised T cell function may significantly reduce the systemic immune function. The collapse of immune cell balance function refers to the nonfunctional state or a state of significantly compromised function of immune cells. In VTE patients, cytological examination reveals that the T cell function is significantly compromised, and genomic analysis indicates that the function

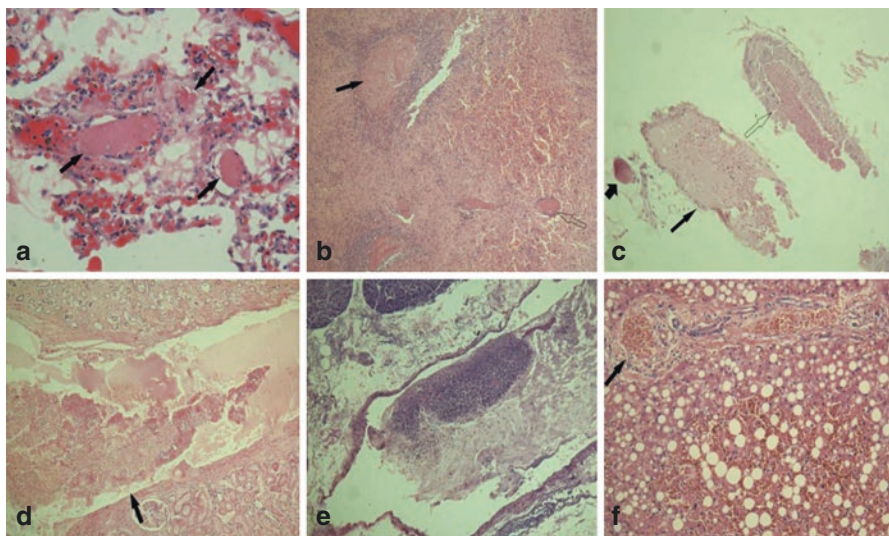


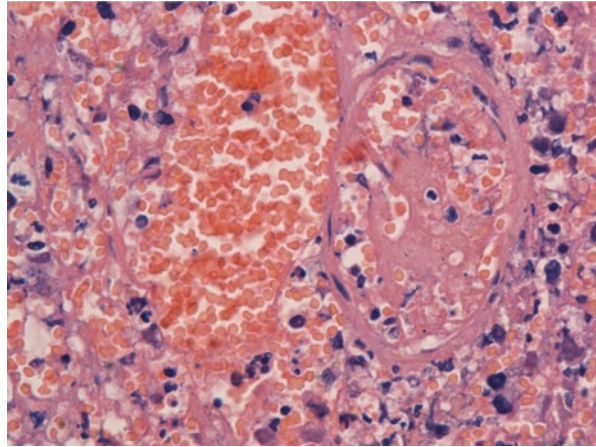
Fig. 1.22 (a–f). HE staining of multiple organs from a SARS patient after autopsy. (a). Lung: thrombi in the lumen of the small vessels (arrow) and significant pulmonary edema and hemorrhage. HE $\times 200$; (b) spleen: thickening and hyaline degeneration of the splenic central artery (solid arrow) and the hyaline thrombi in small vessels (open arrow). HE $\times 40$; (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 $\times 100$; (d) renal interstitium: mixed thrombi (arrow) composed of fibrin, platelets, and red blood cells. HE $\times 40$; (e) pancreatic lobular interstitium: aggregation of white blood cells and fibrin clot, incomplete occlusion in the vascular cavity (arrow), and perivascular interstitial edema. HE $\times 40$; (f) liver: vascular dilatation and congestion in the periportal region are shown (arrow). HE $\times 100$ (Reprinted with permission from *Am J Respir Crit Care Med.* 2010; 182(3): 436–437 [29])

of systemic immune cells is significantly inhibited. These suggest that the immune cells become dysfunctional and present a collapsed state of immune cell balance function.

1.9 VTE Is a Consequence to Defense Against Malignant Cells in Proliferative Phase

Malignancy is a risk factor of VTE, and VTE has been one of the main causes of death in malignant patients [33–35]. The risk for VTE in malignancy patients is 4–7 times higher than in subjects without malignancy [36–38]. The occurrence of malignancy is a consequence of ineffective and delayed clearance of malignant cells by immune cells. We reported that there were necrosis, granulation tissues and rupture of small veins on the sigmoid colon adenocarcinoma, and dark-staining fibrinogen in the vein formed filament-like mesh [13, 14]. In addition, in the necrotic area of poorly differentiated adenocarcinoma of the stomach, a large amount of red cells were observed outside the blood vessels (Fig. 1.23); cancer cells were observed

Fig. 1.23 A large amount of red blood cells outside of blood vessels in the necrotic area of poorly differentiated adenocarcinoma of the stomach (Reprinted with permission from Int J Clin Exp Med 2014; 7: 1319–1323 [14])



around the veins, and dark-staining fibrinogen formed filament-like mesh in which there were a large amount of cancer cells; the filament-like mesh was able to block the hematogenous metastasis of cancer cells. Based on these findings, we speculate that the occurrence of VTE and hemorrhage in malignancy patients is consequent to the invasion of cancer cells into surrounding veins and arteries due to the unlimited proliferation of cancer cells and is a product in the proliferative phase of cancer cells.

1.10 Differentially Expressed Core Proteins in VTE Subjects and in Risk Population of VTE

A total of 1006 subjects were divided into VTE group, risk factor group, and control group, and flow cytometry was employed to detect the core proteins. In healthy controls, the normal range of integrins β_1 , β_2 , and β_3 expression was determined [39].

When compared with control group, the integrin β_1 expression increased significantly in VTE group and INF [acute infection], CAN [cancer], and IMD [autoimmune disease] of risk factor group ($P < 0.001$, $P < 0.01$) but not on surgery and trauma subgroups (SUR) of risk factor group ($P > 0.05$).

When compared with control group, the integrin β_2 expression increased significantly in VTE group but not in INF, CAN, IMD, surgery, and trauma (SUR) subgroups ($P > 0.05$) of risk factor group.

When compared with control group, the integrin β_3 expression increased significantly in VTE group but not in INF, CAN, IMD, surgery, and trauma (SUR) subgroups ($P > 0.05$) of risk factor group (Fig. 1.24).

When compared with control group, the integrins β_1 , β_2 , and β_3 expression increased significantly in VTE group; the integrin β_1 expression in INF, CAN, and IMD subgroups of risk factor group was significantly higher than in control group; the integrins β_1 , β_2 , and β_3 expression in surgery and trauma subgroup (SUR) was

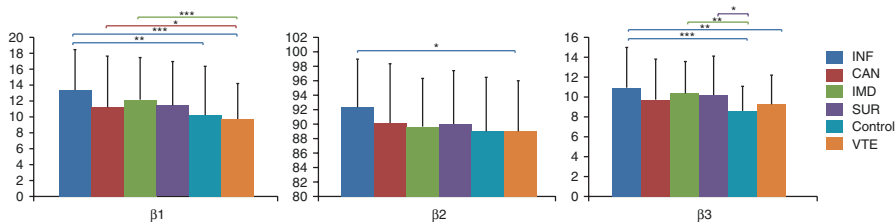


Fig. 1.24 (1) There is significant difference between VTE (INF, CAN, IMD) and control group in integrin β_1 expression, VTE, and SUR group in integrin β_1 expression. (2) There is significant difference between VTE and control groups in integrin β_2 expression. (3) There is significant difference between VTE and control group in integrin β_3 expression and VTE and (SUR) group in integrin β_3 expression. * $P < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Reprinted with permission from Am J Transl Res 2015; 7(3):624–631 [39])

comparable to that in control group ($P > 0.05$). These results showed integrin β_1 is a shared protein between VTE group and risk factor group, and the increase in integrins β_2 and β_3 is a key in the occurrence of VTE; the increase in integrin β_1 is a characteristic of risk factor group, but it remains unchanged in surgery and trauma (SUR) subgroup, which has no increased expression of core protein as in other subgroups of risk factor group. Thus, we propose that surgery and trauma themselves are not the true risk factors of VTE, but the occurrence of VTE in patients with trauma or surgery is temporally consistent with the presence of collapse of immune cell balancing function.

1.11 Core Proteins May Become the Diagnostic Markers of VTE

We investigated the efficacy of integrins β_1 , β_2 , and β_3 expression in the diagnosis of VTE in 120 patients. Our results showed the area under the ROC was 0.870, 0.821, and 0.731 for integrins β_1 , β_2 , and β_3 , respectively, in the diagnosis of acute VTE; the ideal threshold of Youden index was 10.29 pg/mL, 91.10 pg/mL, and 10.35 pg/mL for integrins β_1 , β_2 , and β_3 , respectively. At the ideal threshold of Youden index, the diagnostic sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were 80.3%, 83.7%, 71.1%, and 89.3%, respectively, for integrin β_1 ; 78.6%, 73.7%, 59.4%, and 87.6%, respectively, for integrin β_2 ; and 68.4%, 71.2%, 54.3%, and 81.8%, respectively, for integrin β_3 . When the expression of integrins β_1 , β_2 , and β_3 was employed in the diagnosis of acute VTE, the AUC was as high as 0.916, and the diagnostic sensitivity, specificity, PPV, and NPV were 84.6%, 90.8%, 81.7%, and 92.0%, respectively. Clinical experience indicated that the expression of integrins β_1 , β_2 , and β_3 increased significantly in VTE patients and had high specificity and sensitivity in the diagnosis of VTE (Figs. 1.25, 1.26, 1.27, and 1.28) [40].

The population with the collapse of immune cell balancing function is susceptible to venous thrombosis, and patients with venous thrombus might have cells

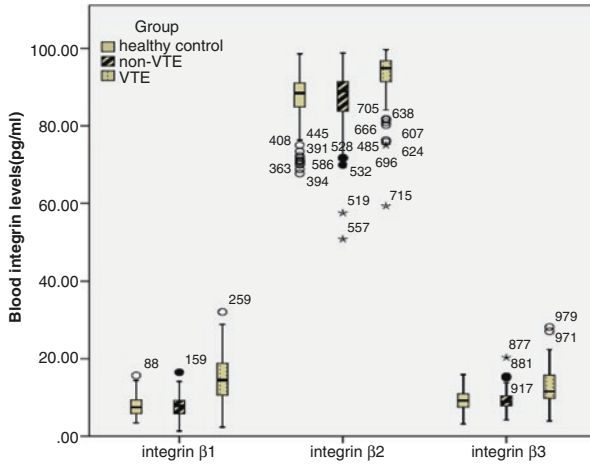


Fig. 1.25 Blood integrins β_1 , β_2 , and β_3 expression in VTE patients, non-VTE patients, and healthy controls. Integrin expression was compared with Mann-Whitney U test. Significant difference in blood integrins β_1 , β_2 , and β_3 expression was observed between VTE patients and non-VTE patients ($P = 0.000$, 0.000 and 0.000 , respectively), and between VTE patients and healthy controls ($P = 0.000$, 0.000 , and 0.000 , respectively). There were no significant difference between non-VTE patients and healthy controls ($P = 0.572$, 0.544 and 0.547 , respectively) (Reprinted with permission from Int J Clin Exp Med 2014; 7: 2578–2584 [40]).

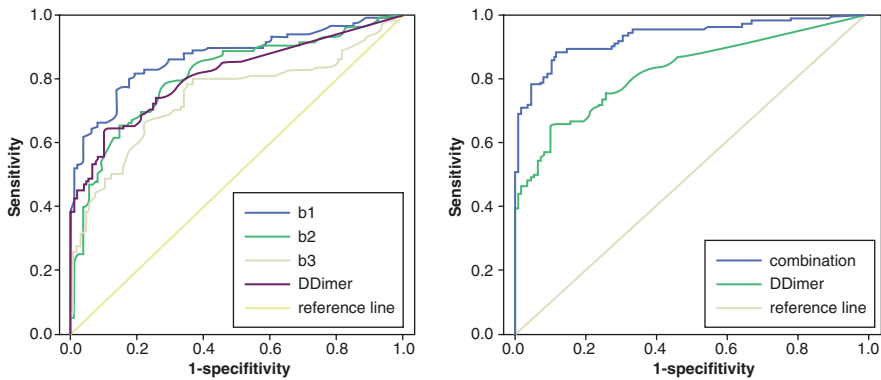


Fig. 1.26 Receiver operating characteristic (ROC) curves for integrins β_1 , β_2 , and β_3 expression in distinguishing VTE patients from non-VTE patients. The comparative ROC curves for three integrins (left) and the combination of three integrins (right) and D-Dimer are provided. The area under the curve (AUC) of integrin β_1 , integrin β_2 , and integrin β_3 was 0.869 ($P = 0.000$, 95%CI, 0.821 – 0.916), 0.809 ($P = 0.000$, 95%CI, 0.752 – 0.867), and 0.742 ($P = 0.000$, 95%CI, 0.676 – 0.809), respectively, and that of combined three integrins and D-Dimer was 0.917 ($P = 0.000$, 95%CI, 0.878 – 0.956) and 0.811 ($P = 0.000$, 95%CI, 0.754 – 0.868), respectively (Reprinted with permission from Int J Clin Exp Med 2014; 7: 2578–2584 [40]).

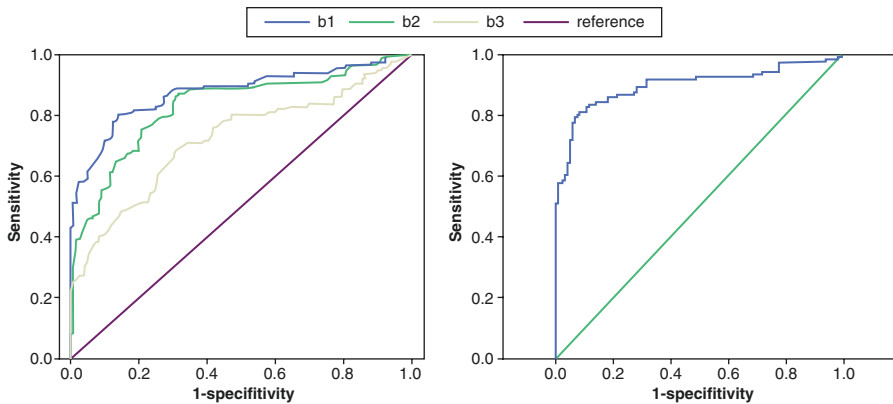


Fig. 1.27 Receiver operating characteristic (ROC) curves for integrins β_1 , β_2 , and β_3 expression in distinguishing VTE patients from healthy controls. The comparative ROC curves for three integrins (left) and the combination of integrins (right) are provided. The area under the curve (AUC) of integrin β_1 , integrin β_2 , and integrin β_3 was 0.875 ($P = 0.000$, 95%CI, 0.829–0.922), 0.828 ($P = 0.000$, 95%CI, 0.774–0.882), and 0.721 ($P = 0.000$, 95%CI, 0.655–0.786), respectively, and that of combined three integrins was 0.915 ($P = 0.000$, 95%CI, 0.876–0.954) (Reprinted with permission from Int J Clin Exp Med 2014; 7: 2578–2584 [40])

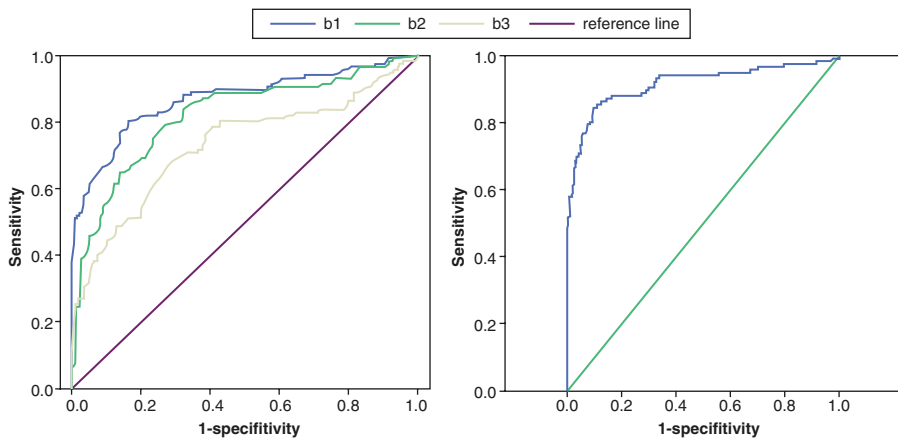


Fig. 1.28 Receiver operating characteristic (ROC) curves for integrins β_1 , β_2 , and β_3 expression in distinguishing VTE patients from non-VTE patients plus healthy controls. The comparative ROC curves for three integrins (left) and the combination of integrins (right) are provided. The area under the curve (AUC) of integrin β_1 , integrin β_2 , and integrin β_3 was 0.870 ($P = 0.000$, 95% CI, 0.825–0.915), 0.821 ($P = 0.000$, 95%CI, 0.771–0.871), and 0.731 ($P = 0.000$, 95%CI, 0.671–0.792), respectively, and that of combined three integrins was 0.916 ($P = 0.000$, 95%CI, 0.878–0.953) (Reprinted with permission from Int J Clin Exp Med 2014; 7: 2578–2584 [40])

with intracellular infection/malignant cells. Advanced age, infection, trauma, surgery, autoimmune disease, pregnancy, delivery, long distance travel syndrome, and family history are the risk factors of VTE. In the presence of collapse of immune cell balance function, venous thrombosis may occur in the subjects with these risk factors. In subjects with normal immune cell balance function, intracellular infection/proliferation of cancer cells usually does not cause VTE even in the presence of risk factors. The core proteins of acute venous thrombus are the integrins β_1 , β_2 , and β_3 , and increase in these proteins was observed in VTE patients. The increase in integrin β_1 alone was a characteristic of patients with risk factors for VTE. The clinical detection of integrins β_1 , β_2 , and β_3 may guide the diagnosis of VTE, the anticoagulation treatment, and the screening of population with risk for VTE [41–42].

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