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# Insights in thrombosis and haemostasis: from a biological, clinical and genetic perspective

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# Insights in thrombosis and haemostasis: from a biological, clinical and genetic perspective

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# Editorial: Insights in thrombosis and haemostasis: from a biological, clinical and genetic perspective

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Editorial on the Research Topic
Insights in thrombosis and haemostasis: from a biological, clinical and genetic perspective

Thrombosis and haemostasis are the two essential physiological properties of blood. Their primary purpose is to prevent bleeding, but even small changes at the molecular level may alter this process, leading to complex pathophysiological alterations that affect both arteries and veins, subsequently resulting in morphological and functional changes in the tributary territories. A detailed clinical evaluation may sometimes indicate the location and severity of embolism/thrombosis, but in most situations, multiple investigations are necessary to establish an accurate diagnosis.

"Insights in Thrombosis and Haemostasis: from a Biological, Clinical and Genetic Perspective" debates aspects of deep vein thrombosis (DVT), starting with several genetic alterations and culminating with peculiar clinical presentations. A second section refers to laboratory investigations capable of revealing the underlying pathways of atherosclerosis and platelet aggregation.

In "Insights in Thrombosis and Haemostasis: from a Biological, Clinical and Genetic Perspective", we present several research studies that have focused on defining the mechanisms responsible for DVT, aiming to improve its prevention. Some studies observed that an elevated blood urea nitrogen and creatinine ratio (BCR) level was independently linked with an increased risk of in-hospital mortality among critically ill patients diagnosed with venous thrombo-embolism (VTE). Given its widespread availability and ease of measurement, BCR could be a valuable tool for risk stratification and prognostic prediction in VTE patients. Other scientists determined that aberrant gene expression, at the level of MicroRNAs, usually 21–23 nucleotides long, can impact

biological functions and influence the development and evolution of cardiovascular and venous diseases. The epigenetic modification of forkhead box protein 3 (FOXP3) at the posttranscriptional level, might be the key trigger leading to the down-regulation of FOXP3 expression in patients with DVT. Overexpressed miR-6132 reduced FOXP3 expression and aggravated DVT formation, while miR-6132 knockdown increased FOXP3 expression and alleviated DVT formation. The microRNAs regulating the expression of FOXP3 might represent novel targets for future DVT treatment options.

Subsequently, we discuss the connection between the development of DVT and physiological conditions (pregnancy), and some predisposing pathologies (especially spinal cord injury). Pregnant women with DVT pose various challenges for diagnosis and treatment. To decrease the risk for both mother and child, we focused on the early detection, diagnosis, and treatment of pregnancy-associated venous thromboembolism by providing an overview of radiological diagnostic techniques in this particular situation. Among the pathologies associated with DVT, cancer, and spinal cord injuries are associated with the worst prognosis for these patients. By screening 232 patients with spinal cord injury and cervical fracture, we observed a high rate of DVT exacerbation. The worsening of existing DVT or the development of new DVT was favoured by older age, ASIA score A-B, time from injury to surgery, surgery time, and blood loss. Surprisingly, several cases of DVT that occurred in younger men (under 40 years old) have been described. Although all experts consider older age a risk factor for DVT, in recent years, an increase in the incidence of lower limb DVT has been detected even in young adults, under 40 years old. Several particularities have been observed: male sex, the frequent involvement of the inferior vena cava, which could at least partially explain the higher incidence of DVT recurrence.

One of the main challenges in patients with DVT is adequate treatment, which starts with nursing care and ends with oral anticoagulants and/or surgical interventions. Nursing care should focus on emphasizing comprehensive preoperative and postoperative evaluations to optimize patient outcomes. These evaluations facilitate tailored treatment plans, crucial for managing the complex needs of DVT patients. Regarding the long-term anticoagulation, most practitioners prefer direct oral anticoagulants, which offer a safer profile and simplified management compared to traditional therapies. Mechanical interventions are also an alternative (balloon angioplasty and venous stenting) as they can improve immediate and long-term vascular function in acute cases. Furthermore, the use of image-guided techniques is essential for enhancing the accuracy and safety of these interventions. The combined use of systemic thrombolysis with Tenecteplase and anticoagulation may be a treatment option for acute extensive portal venous system thrombosis (PVST) in the future. There are no recent studies that examine the association between antifactor-Xa (AFXa) based heparin monitoring and clinical outcomes in the setting of cerebral venous thrombosis (CVT). Although monitoring UFH based on AFXa values may be practical, reaching target AFXa levels within 24 h of hospitalization may not necessarily be prognostic.

In the second part of our topic, we discuss the underlying pathophysiological mechanisms relevant to the occurrence of coronary artery disease (CAD). Inflammation, oxidative stress, and the activation of the sympathetic nervous system form an interconnected network, with each component influencing the others and creating a vicious circle. To better evaluate patients scheduled for bypass surgery, it would be beneficial to develop a scoring system including biological markers indicating inflammation and oxidative stress. This could help us to identify patients that require a more aggressive approach to lower inflammation, oxidative stress, and modulate the sympathetic nervous system. Some studies suggested that oxidative stress and inflammation can be worsened during coronary artery bypass grafting (CABG). Remote ischemic preconditioning (RIPC), a non-invasive approach to protect the heart and other organs from ischemia and reperfusion injury, may improve the outcome of these patients. Patients undergoing RIPC before CABG had reduced plasma levels of Lectin-like oxidized low-density lipoprotein (LDL) receptor-1 (LOX-1) and increasing total values of Superoxide dismutase-1 (SOD-1) after surgery.

Platelet endocytosis was first mentioned in the medical literature in the 1980s when the existence of active internal systems in platelets was first identified. Transmission electron microscopy enabled the observation of platelets internalizing small particles (such as fibrinogen, IgG, and albumin) into membranous structures through a phagocytosis-like mechanism. Platelet endocytosis also has a role in haemostasis and various pathophysiological processes. Platelet endocytosis may be influenced by viral infections, such as the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Platelets in patients with COVID-19 exhibited hyperactivity, but a newer theory suggests that the SARS-CoV-2 virus could be ingested by platelets, as it was demonstrated that the virus could penetrate the platelet surface in the intracellular platelets. Other data supporting this hypothesis are the fact that mRNA traces of SARS-CoV-2 were detected in isolated platelets, and virions could be observed within platelet sections.

Despite the direct effects of the virus on the human body, soon after the development of the first vaccine and its extensive use, several adverse effects were reported. Although rare, one of the most severe ones was clotting and thrombocytopenia events, followed by subsequent coagulation abnormalities, more frequently encountered after adenovirus-DNA-based vaccines (Janssen/Johnson & Johnson and Astra-Zeneca vaccines). A new pathology was defined, the Vaccine-Induced Immune Thrombotic Thrombocytopenia (VITT), characterized by unusual cerebral and splanchnic venous thrombosis, and circulating autoantibodies directed against anti-platelet factor 4 (PF4). VITT has a very low incidence and does not affect the overall benefit of immunization; however, if left untreated, it can be debilitating or even fatal.

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## Author contributions

CT: Investigation, Conceptualization, Methodology, Supervision, Funding acquisition, Writing – review & editing, Software, Formal Analysis, Writing – original draft, Project administration, Visualization, Data curation, Resources, Validation. RD: Methodology, Validation, Visualization, Conceptualization, Writing – original draft, Writing – review & editing. GE: Supervision, Writing – original draft, Writing – review & editing, Data curation, Validation, Conceptualization, Visualization. IT: Writing – review & editing, Writing – original draft. FV-M: Writing – original draft, Supervision, Investigation, Funding acquisition, Writing – review & editing, Software, Formal Analysis, Methodology, Project administration, Data curation, Resources, Visualization, Conceptualization, Validation.

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# FcγRIIa - dependent platelet activation identified in COVID-19 vaccine-induced immune thrombotic thrombocytopenia-, heparin-induced thrombocytopenia, streptokinase- and anisoylated plasminogen-streptokinase activator complex-induced platelet activation

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Coronavirus disease 2019 (COVID-19), which was caused by the coronavirus - severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was globally responsible for remarkable morbidity and mortality. Several highly effective vaccines for COVID-19 were developed and disseminated worldwide within an unprecedented timescale. Rare but dangerous clotting and thrombocytopenia events, and subsequent coagulation abnormalities, have been reported after massive vaccination against SARS-CoV-2. Soon after their global rollout, reports of a morbid clinical syndrome following vaccination with adenovirus-DNA-based vaccines appeared. In the spring of 2021, reports of a novel, rare and morbid clinical syndrome, with clinically devastating and fatal complication after vaccination with adenovirus-based coronavirus vaccines (Janssen/Johnson & Johnson and Astra-Zeneca vaccines) led to a brief suspension of their use by several countries. Those complications were associated with unusual cerebral and splanchnic venous thrombosis, and circulating autoantibodies directed against anti-platelet factor 4 (PF4), a protein secreted from platelets, leading to the designation: Vaccine-Induced Immune Thrombotic Thrombocytopenia (VITT). The reported VITT incidence remains very low and does not affect the overall benefit of immunization, however, if left untreated, VITT can be debilitating or even fatal. VITT resembled specific adverse drugs' reactions that also involved the production of autoantibodies and subsequent abnormal platelet activation through platelet FcγRIIa. These unusual but well-documented drug reactions were heparin-induced thrombocytopenia (HIT), streptokinase- (SK), and anisoylated plasminogen-streptokinase activator complex- (APSAC) associated with platelet-activating antibodies. There was considerable overlapping of clinical features between VITT, COVID-19 and these adverse drugs' reactions. We review the phenomenon of VITT against the backdrop of shared and common mechanisms that underlie HIT-, SK-, and APSAC-platelet FcγRIIa-dependent platelet activation. An understanding of VITT's

pathogenesis may be achieved by comparing and contrasting VITT-, HIT-, SK- and APSAC-induced platelet activation mechanisms, their respective physiopathology and similarities. Discussing these conditions in parallel provides insight into complex immunological disorders and diseases associated with abnormal hemostasis and thrombosis in particular.

KEYWORDS

vaccine-induced immune thrombotic thrombocytopenia, COVID-19, platelet activation, FcγRIIa, heparin-induced thrombocytopenia, vaccination

## Introduction

Coronavirus disease 2019 (COVID-19), which was caused by the coronavirus - severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was globally responsible for remarkable morbidity and mortality. The SARS-CoV-2 virus deploys its "spike protein" in order to gain entry to cells by chemically associating with angiotensin-converting enzyme 2 (ACE2) located on the outer membrane of the cells of the host (1, 2). The transmembrane protease serine 2 (TMPRSS2), a serine protease, enzymatically cleaves and proceeds to activate the spike protein to fuse the SARS-CoV-2 virus with the cell membrane. This spike protein, a uniquely recognizable virus component, became the target for vaccine manufacturers. Several highly effective vaccines were developed for COVID-19. These vaccines were designed to trigger the immune system, thus resulting in the production of neutralizing antibodies with the ability to identify the SARS-CoV-2 virus. European and US regulatory authorities approved two technological approaches to producing and administering COVID-19 vaccines. These were lipid nanoparticles encapsulating an mRNA payload acting as a template for the SARS-CoV-2 spike protein (BioNTech/Pfizer and Moderna), and replication-attenuated adenovirus vectors carrying a DNA payload encoding the same spike protein (Ad26.COV.2.S or Janssen/Johnson & Johnson and ChAdOx1 nCoV-19 or Astra-Zeneca). All available vaccines, whether they employed mRNA or DNA, led to the host cells' expression of the SARS-CoV-2 spike protein and elicitation of protective immunity (3). The adenovirus-DNA-based vaccines became caught up in a controversy that led to their temporary suspension in several countries. Midway through 2021, reports of a novel, rare and morbid syndrome with devastating and fatal complications after vaccination were published. It was associated with atypically located cerebral and splanchnic venous thrombosis, abnormal platelet activation, and circulating autoantibodies, which attach themselves to the platelet-secreted protein, platelet factor 4 (PF4). This adverse reaction was designated vaccine-induced immune thrombotic thrombocytopenia (VITT) (4–8).

The proposed pathological mechanism of VITT was the production of these antibodies that were directed against the naturally occurring human protein PF4 as a reaction to the vaccine. This led to massive platelet aggregation. It is also conceivable that the thrombocytopenia following vaccination had multiple causes. Other factors may have been necessary for the development of VITT. The proteins ACE2 and TMPRSS2 are abundantly expressed in platelets. These membrane proteins are responsible for spike protein priming (9, 10). Therefore, it was no surprise that platelets would have been involved in the clinical manifestations of COVID-19 disease. However, the function played by platelets in this post-vaccine adverse reaction was more complex than mere virus-protein to host-protein interactions.

Platelets are crucial conduits conjoining the hemostatic system and immune defenses. This includes those immunological mechanisms and processes protecting against viral disease. Platelets also serve as mediators involved in vital elements of inflammatory processes and are instrumental in modulating responses of the immune system to both "self" and "foreign" molecules (11, 12). The cellular phenomenon of platelet activation is known for its crucial contributions to thrombus formation. Hence platelet-activation's role in preventing bleeding and minimizing vascular injury. However, along with this physiological role comes a pathological role, played out in deleterious thrombotic events and associated cardiovascular sequelae. Not dissimilar to innate immune cells, platelets express an array of immune-associated molecules. These include receptor molecules for: IgG's Fc-region, C-type lectin and various complement and chemokine molecules. They also express toll-like receptors (TLRs), and nucleotide-binding and oligomerization domain–like receptors (13, 14). These receptors recognize pathogens such as dengue, human immunodeficiency, and influenza viruses. When in contact with pathogens, platelets mediate immune responses indirectly through the release of cytokines, anti-microbial peptides and directly through interaction with neutrophils, monocytes, and lymphocytes. The net result of which is the amplification of the immune response (15). Furthermore platelets possess a plethora of coagulation factors and inflammatory factors. These are stowed in their α-granules, only to be secreted upon platelet activation to boost further the cascade of events that comprise coagulation (16, 17). Factors V and XIII function as sites of adhesion for the binding of coagulation factors activation via their cell surface exposure to phosphatidylserine (18).

The descriptions of VITT are recent, and research on its pathophysiology is limited. Pathogenicity of VITT may involve both host and vaccine factors (19). However, several grey areas remain regarding knowledge of VITT and its pathophysiology. This poses a stark standout question. How could a strand of DNA coding for a viral protein delivered in an adenovirus vector generate autoantibodies against a human physiologically functional protein such as PF4? The answer may lay in adverse drug reactions involving totally different chemical compounds.

The clinical and laboratory details of this adverse reaction to the COVID-19 vaccines revealed intriguing similarities to previously well-documented adverse drug reactions. Platelet activation and the enhancement of platelet activation by autoantibodies through platelet Fc$\gamma$RIIa is a common feature of VITT with remarkable similarities to heparin-induced thrombocytopenia (HIT), streptokinase (SK), and anisoylated plasminogen-streptokinase activator complex (APSAC) associated platelet-activating antibodies through platelet Fc$\gamma$RIIa. Accounting for commonalities between the various responses to these medications may expand our knowledge of the pathogenesis of VITT. Comparing and contrasting elements of the VITT response to a vaccine with these other abnormal responses to drugs may allow us to better understand VITT and the mechanisms underpinning adverse reactions to heparin, SK and APSAC. In order to comprehensively assess VITT, in light of the responses to these drugs, it is necessary to review elements of the pathology of COVID-19, the vaccines, platelet function and the role of platelets in the above-mentioned adverse drug reactions.

## COVID-19 and thrombosis

The putative cause of VITT is anti-PF4 antibodies inducing pathological platelet activation through Fc$\gamma$RIIa, a protein located on the outer membrane of platelets. Circulating immunoglobulin-G antibodies with the ability to induce platelet activation via these Fc$\gamma$RIIa proteins have also been detected in patients with SARS-CoV-2 virus infection. Therefore, this necessitates a brief exploration of some features of COVID-19 itself in relation to VITT.

Thromboembolic complications are prominent clinical features of COVID-19 (20). Thrombosis in atypical locations, such as cerebral veins, has been reported in patients afflicted with COVID-19. A notable overlap exists in the clinical presentation between VITT and COVID-19, i.e., unusual thrombotic complications have been associated with patients with both conditions. Less severe thrombocytopenia occurs frequently with patients with severe COVID-19 with or without it being leagued with hemostasis activation and systemic inflammation, including fibrinolytic shutdown (21, 22). Substantial proinflammatory aspects, indicate a so-called "cytokine storm", may often occur with COVID-19 patients. Thus being implicated in the pro-thrombotic syndrome (23). Thrombocytopenia occurred more often in patients with the more severe forms of COVID-19 (24). Twenty per cent of patients with COVID-19 had low platelet counts at the time of hospital admission (25). Among survivors of COVID-19, blood platelet counts were inclined to increase within the second week of hospitalization, whereas, among non-survivors, low blood platelet counts were more inclined to remain low or worsen (26). There is a body of evidence that suggests that COVID-19 predisposes individuals to thromboembolic complications, including arterial and venous thrombotic events, as well as thrombocytopenia. These have all been significantly associated with mortality (27). Reports from several independent research groups described platelets as being hyperreactive during COVID-19 disease, this being coupled with abnormalities of gene expression in platelets. This potentially influences interactions between white blood cells and platelets (28). Quiescent platelets isolated from the blood of patients with COVID-19 had abnormally high expression of P-selectin upon activation. These patients also had raised levels of circulating platelet-monocyte, platelet-neutrophil, and platelet-T-cell aggregates (29–31). Other markers of thrombin generation or activation have been associated with COVID-19. These include thrombin-antithrombin complex as well as prothrombin fragments (32).

The immune system's responses to PF4 involve separate mechanisms from the immune responses to the spike protein of SARS-CoV-2 virus particle. Further, VITT antibodies have been shown not to bind to the spike protein of SARS-CoV-2. This suggests that the response of the immune system to the SARS-CoV-2 spike protein in the vaccine does not induce VITT (33). When discussing VITT, the intended and non-intended immune responses to the vaccine should not be conflated with the immune response to the viral infection of SAR-CoV-2. However, due to the shared and common elements, discussions of the pathogenesis of VITT are here addressed in parallel with issues related to COVID-19 and the responses to specific drugs.

## Vaccine-induced immune thrombotic thrombocytopenia (VITT)

Clinically, VITT is associated with complications related to the hemostatic system (**Tables 1**, **2**). These may be life-threatening thromboses such as deep venous thrombosis, cerebral venous sinus thrombosis, and splanchnic vein thrombosis. These thrombotic events occur paralleled with thrombocytopenia, elevated plasma soluble D-dimer concentrations and raised numbers of activated platelets. Also, there is a detectable amount

TABLE 1 Most common complications associated with VITT.

| Most common complications associated with VITT | References |
|---|---|
| Aortic graft thrombosis. | Giacomelli E et al., (34) |
| Catheter-associated upper-limb DVT. | Bozzani A et al., (35) |
| Cerebral venous sinus thrombosis (CVST), splanchnic vein thrombosis, pulmonary embolism, deep vein thrombosis, arterial thrombosis, ophthalmic vein thrombosis, intraparenchymal brain hemorrhage, ischemic stroke, and DIC. | Greinacher A et al., (4) Perry RJ et al., (36) de Barry O et al., (37) Hughes C et al., (38) Hemasian H et al., (39) Klein DE et al., (40) Bayas et al., (41) |
| Other forms of thrombosis, especially venous thromboembolism, abdominal vein clots, and arterial clots. | Rizk JG et al., (42) |
| CVST, estimated to occur at a rate of 1 per 100 000 vaccine recipients with the Astra-Zeneca vaccine and 1 per 1 000 000 with the Janssen/Johnson & Johnson vaccine. In the US, all patients who received the Janssen/Johnson & Johnson vaccine and developed CVST with VITT were female. | Ropper AH & Klein JP. (43) |

TABLE 2 Abnormal laboratory and radiologic abnormalities occurring in VITT.

| Abnormal laboratory and radiologic abnormalities occurring in VITT | References |
|---|---|
| ■ The development of thrombosis at uncommon sites includes CSVT/ splanchnic venous thrombosis. It is unclear why this immune-mediated thrombosis manifests mainly in the cerebral vessels and splanchnic circulation.<br>■ Presence of VITT autoantibodies, although VITT patients have not received heparin and it is likely that an unidentified polyanion in the adenoviral vaccines or expressed by the infected cells by the vaccine is binding to PF4. In patients with suspected or confirmed VITT, rapid initiation of treatment similar to that of severe HIT is hence recommended.<br>■ Mild to severe thrombocytopenia. However, a normal platelet count does not exclude the possibility of VITT in its early stages.<br>■ Positive antibodies against PF4 identified by enzyme-linked immunosorbent assay (ELISA) assay. Serologic studies do not show antigen cross-reactivity between anti–SARS-CoV-2 and anti-PF4 antibodies, signifying that anti-PF4 antibodies are unlikely a byproduct of anti-SARS-CoV-2 immunity. Demonstration of circulating anti-PF4 antibodies in conjunction with thrombocytopenia and thrombosis suggests that VITT is a clinical variant of HIT. | Greinacher A et al., (4) Sadoff J et al., (44) Schultz NH et al., (7) See I et al., (8) Oldenburg J et al., (45) Scully M et al., (46) |

of anti-PF4-polyanion immunoglobulin antibodies in serum (47, 48). European studies showed an incidence of around 1 per 100,000–250,000 vaccine recipients. More than 90% of these patients were below the age of 60 years and disproportionately female. Fatalities among these VITT patients were reported to be approximately 20%. This relatively low figure may be due to a lag in recognizing and identifying the symptoms and signs by patients and healthcare providers (49).

HIT and the severe form of COVID-19 disease share several common clinical similarities. These two conditions only occur in a small minority of patients infected with SARS-CoV-2 or those patients exposed to heparin. Dissimilar to the related HIT, with an estimated incidence of 1 to 6% of individuals anticoagulated with heparin and a corresponding mortality rate of 25% (50). VITT has been reported as 1:150,000 of Astra-Zeneca recipients and 1:470,000 of Janssen/Johnson & Johnson recipients. These figures carry a mortality rate of 20%–30% for Astra-Zeneca and Janssen/Johnson & Johnson vaccines respectively (51). HIT and VITT are associated with hypercoagulable blood of affected patients and a relatively high occurrence of thrombosis and thrombotic events within atypical blood vessel sites (21). The twain are associated with abnormal full blood cell count values (particularly leukocytes and platelets), coagulation, prothrombin-time, soluble D-dimer plasma concentrations outside the reference intervals, and a potential for disseminated intravascular coagulation. Similar to HIT, VITT has been associated with developing anti-PF4 antibodies. The typical sequela of which may be aggregation of neutrophils and platelets (52).

## Antibodies against PF4 characterize VITT

Dissimilar to thrombocytopenia caused by heparin exposure, VITT pathological antibodies are produced independently of exposure to this thrombolytic drug. There are no reports that any of the VITT patients had been previously anticoagulated with heparin prior to the onset of VITT. This suggests that a factor in the vaccine preparation triggered the adverse reaction to the vaccine (45, 53). VITT shares many clinical, laboratory and immunological features with HIT, a pro-thrombotic pathology caused by antibodies that induce platelet-activation by recognizing multimolecular complexes. These structures form between cationic PF4 and anionic heparin (see **Figure 1**). These features include thrombocytopenia, unusual strokes, anti-PF4 autoantibodies, and platelet activation through platelet Fc$\gamma$RIIa receptor.

PF4, a 70-amino-acid protein located in α-granules, is secreted from platelets when activated during the aggregation process and promotes further thrombogenesis. PF4 is cationic in its physical nature and acts as a chemokine (54).. Although the primary function of PF4 is to act as a pro-coagulant factor, it is also plays a role in innate immunological and adaptive immunological processes where thrombocytes are activated. This platelet activation is in response to infections. The positively charged PF4 may readily associate with negatively charged bacterial surfaces,

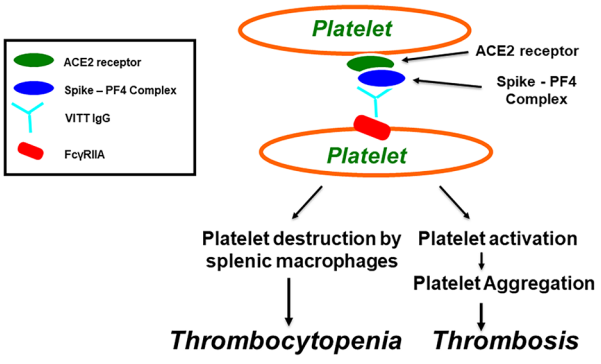


FIGURE 1
Vaccine-induced immune thrombotic thrombocytopenia (VITT). Antibodies induce platelet aggregation through Fc$\gamma$RIIa. SARS-CoV-2 virions, or its spike protein, produced after COVID-19 vaccination, bind to platelets via ACE2 receptor, leading to activation of platelets and the secretion of platelet factor 4 (PF4). PF4 then biochemically associates with the spike protein, forming PF4-Spike complexes that stimulate VITT anti-PF4 antibody production. VITT pathological IgG antibodies induce platelet aggregation through platelet Fc$\gamma$RIIa and thrombocytopenia through platelet destruction by splenic macrophages.

thereby promoting, opsonization and contributing defenses against potentially pathological bacterial infection (55). PF4 also forms molecular associations with polyanions, undergoing a structural change in its conformation, which induces anti-PF4/polyanion antibodies (see **Figure 1**). These help eliminate pathogens (56) and cause HIT in the absence of heparin molecules. This response is termed "spontaneous HIT" (57).

Because VITT patients were not previously exposed to heparin anticoagulation, it is reasonable to suspect a similar mechanism to "spontaneous HIT". *In vitro* experiments demonstrated that proteolysis of plasma proteins leads to sequential release of endogenous glycosaminoglycans, chondroitin sulfate and heparin (43). The dysregulated proteolysis occurring during COVID-19 or VITT triggers a sustained and excessive release of endogenous chondroitin sulfate and/or heparin that may play a fundamental role in their associated and respective pathophysiology (43). Platelets stimulated by endogenous heparin, either directly or with the intermediation of PF4, release additional heparin may lead to the catastrophic thrombotic thrombocytopenia observed in severe COVID-19 or following vaccination (43).

The pathophysiology of VITT is presumably due to the development of antibodies against PF4 that associates with a highly restricted molecular location on the PF4 protein. This may correspond to the PF4-heparin-binding site. The result of which may be platelet consumption and depletion accompanied by thrombus formation (4, 58). Several published studies have described high levels of pathological VITT anti-PF4 antibodies, present in blood specimens from VITT patients, which induce the activation of platelets through platelet Fc$\gamma$RIIa receptors in the presence of PF4 without heparin and potentially other cells, through Fc$\gamma$RIIa receptors (4, 7, 46). The platelet-activating effects of VITT anti-PF4 antibodies can be completely inhibited by anti-Fc$\gamma$RIIa monoclonal antibodies (59–61). The presence of high concentrations of polyclonal immunoglobulins may interfere with the platelet-activating effects of these autoimmune anti-PF4 antibodies. This may be interpreted as a confirmation of the immune system's role in this hyper-activation of platelets working via Fc$\gamma$RIIa (61).

There remains the very real possibility that platelet activation contributing to the pathology of VITT may not be solely attributed to anti-PF4 antibodies. Other factors may underpin the processes resulting in these thrombotic events. The progression of HIT may be ascribed to the many pro-thrombotic mechanisms associated with VITT. These include monocyte activation, over production of tissue factor, and pro-coagulant microparticle generation (58). Restricted to eight amino acid sequences that overlap with the heparin-binding site, VITT antibodies associate with molecular regions similar to PF4 as with heparin (58). Anti VITT antibodies may mimic the physiological function of heparin. Thus, assisting in the clustering of the tetramers of the PF4 protein. These are the building blocks of the immune complexes, which bring together Fc$\gamma$RIIa membrane proteins and induce the activation of platelets (58). It is likely that these VITT pathogenic antibodies associate with and activate other non-platelet cells that also express the mildly ubiquitous Fc$\gamma$RIIa. Noteworthy are endothelial cells as well as the leucocytes (60). Molecular polymorphisms of Fc$\gamma$RIIa may influence the risk of VITT and the development of new thrombotic events. These Fc$\gamma$RIIa polymorphisms impact the affinity of association to the various human IgG subclasses and other molecules involved in Fc$\gamma$RIIa transmembrane signaling. A similar discussion of Fc$\gamma$RIIa polymorphisms applies wholly to HIT (62, 63).

It is now well-established that adenovirus engages with and activate platelets (64, 65). Several viruses can directly lead to platelet hyperactivity (66, 67). Further, constituents of Ad-vector COVID-19 vaccines may play the role of "co-factor" binding to PF4, inducing newly formed immunogenic epitopes that potentially induce the production of the pathological anti-PF4 immunoglobulins. The immunoreactivity observed between the vaccine components and platelets or the PF4 protein may be considered a contributor to the genesis and progression of VITT (4, 7). Platelets may be directly be infected by RNA contained within viruses. Therefore RNA from the virus particles may be translated into spike protein via the protein biosynthesis mechanisms possessed by platelets. Thus, initiating an immune response against the body's own platelets (68). Platelets can translate mRNA and synthesize proteins of viral origin, as is seen with dengue, human immunodeficiency, and influenza viruses (69).

## SARS-CoV-2 spike protein chemically associates directly and increases the activation of platelets

The virus SARS-CoV-2 uses receptors in order to infect lung epithelial cells. COVID-19 patients in a critical condition expressing ACE2 as well as TMPRSS2, have hyperactive platelets, mean platelet volume above their reference interval, increased activation of αIIbβ3 and P-selectin expression on thrombocytes (10, 30). MAPK pathway, downstream of ACE2 mediates the potentiating role of SARS-CoV-2 on platelet activation (29). Neutrophils isolated from severe COVID-19 patients demonstrate a clear association with IgG immune complexes. Patients' sera with severe COVID-19 contain high amounts of immune complexes. These complexes potentially activate neutrophils via a mechanism that is partially independent of Fc$\gamma$RIIa platelets (70).

The Spike protein of the SARS-CoV-2 virus particle binds directly to ACE2 on the surface of platelets, enhancing platelet activation and causing the release of coagulation factors and inflammatory cytokines. In addition, this causes the formation of leukocyte–platelet aggregates and induces thrombus formation (10, 28, 30). Like SARS-CoV-2, through this chemical association with ACE2, the spike protein of SARS-CoV-2 stimulates the secretion of PF4 from platelets and activates cells of the immune system to initiate the inflammatory reaction, thus triggering the production of anti-PF4 antibodies (71).

Structural similarities have been evidenced between platelet factor 4 and SARS-CoV-2′s spike protein (4, 72). The chains are similar in their molecular structural organization, including anti-parallel β-pleated sheets uncased within a pair of α-helices. They both possess common amino acid sequences corresponding to

323–335 residues in RBD and 15–27 amino acidic residues in PF4 (72). Additional molecular similarities between the spike and PF4 have been reported upon, either in the RBD or the other spike protein domains (4). The anti-Spike IgG antibodies do not recognize PF4; the anti-PF4 antibodies demonstrate immunoreactivity with "Spike-RBD" (72). In a similar fashion to heparin-PF4 complexes, since RBD and PF4 can interact with a significant affinity, they form PF4-spike complexes that bind to platelet ACE2 receptors that could have an involvement in the immune production of anti-PF4 antibodies, leading to platelets aggregation through platelet Fc$\gamma$RIIa (72).

There also remains the possibility that other receptor proteins mediate SARS-CoV-2 spike-platelet interactions. Heparin sulphate, located on the cell surface, is implicated as a binding site for viruses (73). The molecular association of the SARS-CoV-2′s spike protein to cell membrane-bound heparin sulphate was attenuated in the presence of heparin. Thus hindering the passage of viral particles into host cells (74). A substitution mutation within the spike protein at residue 614 (aspartic acid to glycine) has been identified and suggested to be a clear "fitness advantage" in increasing SARS-CoV-2′s transmissibility in The Americas and Europe (75). Platelets interact with the spike protein of SARS-CoV-2 through various C-type lectin receptor proteins and proteoglycans. Variants generated through alternative-splicing of the spike protein exist, as well as for CD147. This being a transmembrane protein highly expressed in blood cells, including platelets. Further, adenovirus vectors also interact with the CD46 receptor.

## VITT: laboratory assessment, prevention, management, and treatment

The reported laboratory aspects associated with VITT point to a consumptive coagulopathy. This involved thrombocytopenia, low plasma fibrinogen concentrations, and elevated plasma concentrations of D-dimer. This was reported with the severest of HIT cases (76). Most reports strongly correlate VITT with the strong presence of anti-platelet factor 4 in serum and platelet activation as assessed by function laboratory assays (4, 7).

Initial testing should be performed for recently vaccinated patients (i.e., between 4 and 30 days post-vaccination) presenting clinically and with new laboratory findings, including a full blood count, soluble D-dimer measures, and plasma fibrinogen measures. Deviations from reference intervals would identify patients with severe manifestations of VITT. New clinical symptoms may include nausea, vomiting, headaches, vision problems, pain in the abdomen and chest, as well as dyspnea (49). If lab findings are within the reference intervals, then there is a low likelihood of VITT. However, these VITT patients should be continually monitored, and laboratory investigations should be repeated if symptoms reoccur or persist. Suppose there is solely a single isolated incident of thrombocytopenia, with plasma soluble D-dimer and plasma fibrinogen within their reference intervals and an absence of thrombosis. In that case, vaccine-induced ITP should be considered a probable diagnosis, particularly when the index patient had received one of the vaccines containing mRNA, supposing there is suggestive evidence of consumptive coagulopathy (e.g., thrombocytopenia and plasma soluble D-dimer) (49). In that case, diagnostic imaging investigations and anti-PF4 IgG immunoassays should be performed to document VITT. If diagnostic imaging investigations reveal thrombosis, hospitalization and the initiation of clinical interventions involving non-heparin anticoagulant drugs are recommended without the transfusion of platelets (49). For complications with hemorrhaging, intravenous immunoglobulin (IVIG) and the steroid prednisone are suggested to resolve the low platelet counts, cryoprecipitate and fresh frozen plasma should be administered to manage coagulopathy (49).

There is sufficient evidence of common clinical and laboratory characteristics observed between VITT and HIT to adopt similar management approaches to the two syndromes (**Table 3**). Although the pathogenesis of VITT is not yet clear, recently published findings were consistent in describing that the three phenomena of thromboembolic events, low platelet count, and the detection of anti-PF4 antibodies appear as the hallmarks of VITT (9). Society of Thrombosis and Haemostasis Research suggested screening for anti-PF4 antibodies if thromboembolic events or low platelet counts occurs within two weeks post-vaccination (38). Treatment recommendations issued by The American Society of Hematology for VITT are similar to those for severe HIT in patients with low platelet count, thrombosis, and the positive detection PF4-heparin IgG as assessed by immunoassay (38). The regimens for successful management of HIT of autoimmune origin can also be similarly applied to VITT. Interventions may include IVIG and glucocorticoids given in high doses (42).

## Platelet Fc$\gamma$RIIa activation associated with HIT

Both VITT and HIT disorders are associated with thrombocytopenia, thrombosis, the presence of autoantibodies to PF4, and platelet activation through platelet Fc$\gamma$RIIa. The key adverse drug reaction to the anticoagulant heparin is HIT. It is a pharmacologically induced immunoglobulin-mediated thrombocytopenic disorder associated with both arterial and venous thrombosis resulting from the generation of platelet thrombi (84, 85). Approximately 5% of patients who receive heparin anticoagulation therapy develop HIT. Out of those patients, about 5%–80% develop thrombosis, which can involve arterial vessels, venous vessels, or both vessel types (86–88). HIT is characterized by the presence of platelet-activating antibodies that recognize multimolecular complexes between the cationic PF4 and the anionic heparin, leading to platelet activation through Fc$\gamma$RIIa receptors, resulting in thrombosis, endothelial cell injury, and the release of procoagulant-rich microparticles (see **Figure 2**) (89–93). The removal of platelets via phagocytosis by splenic macrophages, in a Fc$\gamma$RIIa-dependent mechanism, or platelet consumption caused by thrombi formation, can explain thrombocytopenia (4, 94).

TABLE 3 VITT: Management and treatment.

| Management of VITT | References |
|---|---|
| Urgent administration of high dose IVIG 1 gram/kg daily for two days. IVIG contains immunoglobulin G of all subclasses derived from pooled human plasma, and has been used in the management of COVID-19. In high doses, IVIG can competitively inhibit the binding of VITT antibodies with the platelet FcγRIIA, thereby inhibiting platelet activation and aggregation. Case series have documented that IVIG improves platelet count and recovery in VITT by inhibition of serum-induced platelet activation. | Greinacher A et al., (4).<br>Schultz NH et al., (7)<br>Bourguignon A et al., (77)<br>Rizk JG et al., (78) |
| ■ Evaluation for HIT/VITT should be performed before administering IVIG to prevent false-negative test results due to potential interference of IVIG with ELISA and platelet activation assays.<br>■ Given its close resemblance to HIT, the use of heparin may be harmful, and non-heparin anticoagulants should be considered such as: direct thrombin inhibitors: Argatroban or bivalirudin, direct oral anti-Xa inhibitors without heparin bridge: Edoxaban, apixaban, dabigatran or rivaroxaban, low molecular weight heparinoid devoid of heparin: Danaparoid, and selective factor Xa inhibitor: Fondaparinux.<br>Restricting catheter-directed fibrinolysis to experienced centers and patients whose condition deteriorates despite intensive coagulation. Systematic reviews have shown that fibrinolytic treatment is associated with intracranial and extracranial major bleeding complications. Thus, fibrinolytic therapy should only be considered in patients with extensive CVST whose condition worsens despite anticoagulation. | Saposnik G et al., (79) Canhão P et al., (80) |
| Other treatment recommendations for VITT<br>■ Hematology specialist consultation must be sought as early as possible.<br>■ IVIG and non-heparin-based anticoagulation should be initiated if there is a high suspicion for the diagnosis of VITT and PF4-ELISA results are pending.<br>■ Platelet transfusions should be avoided and should be considered only if clinically indicated and at the advice of a hematologist.<br>■ Fibrinogen must be corrected with fibrinogen concentrate or cryoprecipitate to maintain a fibrinogen level of >1.5 g/L.<br>■ All forms of heparin that include heparin-based flushes must be avoided until VITT is ruled out.<br>■ Patients should be transferred to a tertiary care center once the diagnosis of VITT is confirmed.<br>■ Both the BSH and ASH recommend continuing systemic anticoagulation for a minimum of three months in patients with documented thrombosis.<br>■ Steroids can inhibit the synthesis of new autoantibodies, thereby interrupting platelet activation and thrombosis. More recently, systemic steroids have been used in combination with IVIG for the management of VITT. Of the 5 individuals with VITT in the study by Schultz et al., 4 were treated with concomitant methylprednisolone (1 mg/kg/d) and IVIG. Three patients had documented CVST and 2 of these patients died. George et al., reported successful treatment for a patient with VITT and CVST using steroids, IVIG, and bivalirudin.<br>Antiplatelet therapies may be warranted while therapeutic intervention targeting the cytokine storm in severe COVID-19 is gaining increasing attention, the use of antiplatelet therapy also warrants further study in treating COVID-19 patients to improve patient outcome. Aspirin administration has been associated with a reduced risk of mechanical ventilation, intensive care unit admission, and in-hospital mortality in 412 hospitalized COVID-19 patients. | Schultz NH et al., (7) Manne BK et al., (30)<br>George G et al., (81) Mehta P et al., (82)<br>Chow JH et al., (83) |

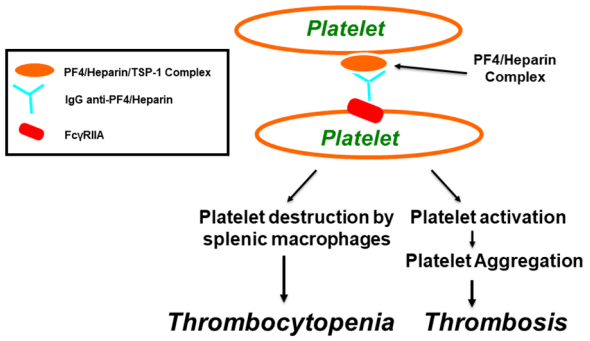


FIGURE 2
Pathogenesis of heparin-induced thrombocytopenia (HIT) as well as thrombosis through FcγRIIa. Within α-granules of platelets, PF4 is associated with a proteoglycan carrier that contains chondroitin-4-sulfate. Platelet factor 4 biochemically associates with physiologic concentrations of heparin, resulting in the molecular formation of the PF4/heparin combined complexes that leads to generation of anti-PF4/heparin antibodies that lead to the generation of anti-PF4/heparin antibodies. Multimolecular complexes of PF4-heparin and antibodies lead to cross-linking of FcγRIIA, inducing platelet aggregation and thrombocytopenia, through platelet destruction by splenic macrophages.

Thrombotic risk in HIT is strongly correlated with high levels of circulating anti-PF4 antibodies, as detected by immunoassays and functional platelet activation assays (95–97). Platelet activation induced by antibody-PF4/heparin complexes is accompanied by other cascading reactions that result in thrombotic complications (98). HIT immune complexes also activate other cells, such as monocytes (99), neutrophils (100), and endothelium (89, 101). HIT antibodies cross-link FcγRIIa on platelets, monocytes, and neutrophils, initiating pro-coagulant cellular responses that generate a profound hypercoagulable state (102). Although HIT is considered a platelet activation disorder, neutrophilia is common, particularly in thrombosis patients (103).

## Platelet FcγRIIa activation associated with SK and APSAC

Platelet activation and the enhancement of platelet activation by autoantibodies binding to platelet membrane FcγRIIa is a common feature of VITT (47), HIT (4), SK, and APSAC (59, 104–107). Both SK and APSAC thrombolytic drugs act predominantly by bringing about the biomolecular transformation of inactive plasminogen to physiologically and biochemically active plasmin. This, in turn, breaks down the physicochemical structures of both fibrin molecules and fibrinogen molecules, thus achieving thrombolysis.

Streptokinase, an exogenous plasminogen activator derived from bacteria, has the latent potential to trigger an innate immune response, subsequently producing antibodies against SK. These anti-SK antibodies inhibit the breakdown of fibrin whilst

simultaneously inducing platelet activation. These antibodies are highly prevalent among particular populations, such as individuals exposed to β-hemolytic streptococci infection or those with previous pharmacological exposure to SK (108). Following SK exposure, there is a progressive increase in detectable plasma levels of anti-SK antibodies, reaching a maximum at around two weeks post-SK thrombolytic therapy (109).

Both SK and APSAC modify the *in vitro* platelet aggregation predominantly via two main mechanisms. These being fibrinogen breakdown (107) and immune responses (see **Figure 3**) (59, 104–107). When SK or APSAC are added to normal subjects' platelet-rich plasma (PRP), there is an inhibition of "platelet aggregation" by way of response to platelet-activating pharmacological agonists [e.g., adenosine diphosphate (ADP) and collagen] (104, 106, 110). The SK or APSAC inhibition effect is mediated by plasmin generation, as it is attenuated in the presence of aprotinin (110). Platelet aggregation inhibition by SK is caused by the fibrinogen fragment E (110). Using PRP from healthy blood donors, the prevalence of SK- (or APSAC) reducing the aggregation of platelets by ADP was found to be 47% (104).

SK or APSAC also potentially induce platelet aggregation via specific circulating anti-SK IgG antibodies (104, 107) (see **Figure 3**). A previous infection with streptococcus or a history of treatment with SK (or APSAC) may influence the presence or absence of a patient's circulating anti-SK antibodies. Awareness of the broader deleterious effects of SK and APSAC may prove paramount when selecting from the array of available thrombolytic therapies so as to minimize the risk of excessive blood loss or thrombosis (104).

Using the PRP collected from healthy donors, we previously reported 5.8% and 53% prevalences for thrombolytic-associated "platelet aggregation" for streptokinase and APSAC, respectively (104). This thrombolytic drug-associated "platelet aggregation" reaction was accompanied by the release of a relatively large concentration of 4.5 μmol/L ATP. However, without streptokinase-induced "platelet aggregation", activation of platelets occurred to some extent with SK-induced platelet shape change only with the release of a mere 0.05 μmol/L ATP (104). SK and APSAC-inducing platelet aggregation or enhancing of platelet aggregation are unaffected by the presence of aprotinin, indicating that the observed effect is specifically associated with SK and works independently of the generation of plasmin, a product from its plasminogen precursor (104). In PRP, SK- or APSAC-induced platelet aggregation showed no discernable correlation with the amount of healthy subject plasma anti-SK antibodies (104). A higher titre of anti-streptokinase antibodies was bereft of any such association with platelet aggregation in response to SK or APSAC exposure, using *in vitro* experimental settings, suggesting that anti-SK antibodies- activating platelets constitute a clearly defined sub-group of whole anti-SK antibodies in plasma (104).

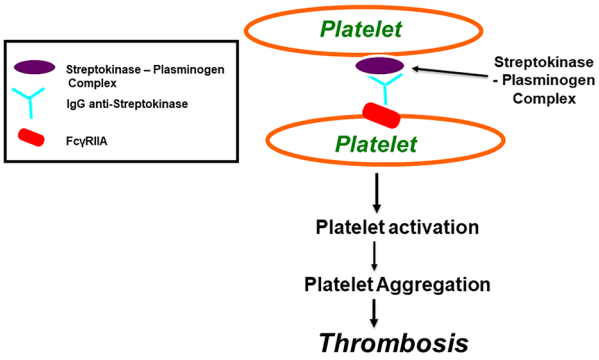


FIGURE 3
Streptokinase (SK) and Anisoylated Plasminogen-Streptokinase Activator Complex (APSAC) induced platelet aggregation through FcγRIIa. Both Streptokinase and APSAC modify in vitro platelet aggregation by two mechanisms; reduced aggregation due to fibrinogenolysis, and enhanced aggregation via an immunological reaction. The reduced aggregation by SK (or APSAC) is mediated by plasmin generation and the fibrinogen degradation product, fragment E. As shown in this figure, SK (or APSAC) may also trigger platelet aggregation by a mechanism involving specific IgG anti-SK. Both SK- (or APSAC) induced platelet aggregation and SK- (or APSAC) enhanced ADP-induced platelet aggregation require the interaction of the Fc domain of the anti-SK antibodies with the platelet FcγRIIA.

Platelets perform a pivotal part in stimulating the response of the immune system in bringing about the said thrombolytic drug-induced "platelet aggregation" in the SK- (or APSAC) "enhanced ADP-induced platelet aggregation" as demonstrated by our in vitro experiments (59, 107). This was evidenced by mixing IgG anti-SK, isolated from patients recently diagnosed with an acute myocardial infarction who received SK for thrombolysis, and washed platelets from apparently healthy volunteer donors (59, 107). The combination of platelets from healthy subjects, "responders" to platelet-activating monoclonal antibody PL2-49, with SK (or APSAC) and purified IgG-induced platelets aggregation whilst simultaneously enhancing the SK platelet aggregation response to ADP. This response is mediated via platelet FcγRIIa and accompanied by the generation of inositol triphosphate, the subsequent cellular influx of $Ca^{2+}$, and TXA2 release (59, 107). Further, compounds known to inhibit the catalytic activities of protein tyrosine kinase (PTK), as well as the enzyme protein kinase-C (PKC) and the biological catalyst phospholipase-C (PLC), have been employed to investigate "platelet aggregation" as induced by "anti-SK antibodies" and additional APSAC in isolated washed platelets. These compounds being: erbstatin - a PTK inhibitor; GF 109203X - a PCK inhibitor; and neomycin – an inhibitor of PLC. The presence of these enzyme inhibitors prevented the aggregation of platelets induced by "anti-SK antibodies" in addition to APSAC, suggesting that PTK, PCK and PCL pathways are fundamental to activating platelets via the immune system (59). The TXA2 receptor antagonist – SQ 29548 and the cyclooxygenase inhibitor – aspirin significantly attenuated the activation of platelets in this *in vitro* experimental setting (59). The release of ADP also contributed considerably to the "platelet aggregation" response, as confirmed by employing apyrase, a compound known to attenuate the immunoglobulin-G anti-streptokinase with the addition of APSAC-induced aggregation of platelets (59).

Two distinct pathways and their dynamic interactions enable "anti-SK antibodies" to elicit streptokinase-induced "platelet aggregation" and "streptokinase-enhanced platelet aggregation". These pathways are: (i) the activation of platelets by the immune system via membrane-bound FcγRIIa, and (ii) the involvement of

a demarcated subgroup of thrombocytes eliciting antibodies directed against SK (59, 104–107).

FcγRIIa structural polymorphisms correlate with platelet's functional response following stimulation and activation by antibodies of mouse origin or aggregated in response to antibodies of human origin (111). Several monoclonal antibodies induce the activation of platelets via interactions between their Fc-domains and membrane FcγRIIa. Platelets from apparently healthy donors demonstrate a vast variation in response to activating monoclonal antibodies (112–116). Although the mechanisms involved in these response disparities have not been definitively elucidated, the majority of reports addressing the platelet FcγRIIa functional polymorphism were performed in PRP (112, 113, 116, 117).

In our previous PRP studies, all platelets from subjects with SK- (or APSAC) elicited aggregation of platelets and SK- (or APSAC) enhanced "platelet aggregation" induced were assigned as "responders" in response to the monoclonal activating antibody PL-249 (88, 89). However, all subjects with only streptokinase (or APSAC) enhanced platelet aggregation were considered "intermediate-responders" and as an alternative group, "non-responders" in the biological reaction of these cohorts to the PL-249 (88, 89). However, in washed platelets, SK- (or APSAC) induced platelet aggregation and SK- (or APSAC-) "enhanced ADP-induced platelet aggregation", observed using platelets from "responders", "intermediate-responders" or "non-responders" to activating monoclonal antibody PL-249, indicating that the functional platelet FcγRIIa polymorphism is observed only when platelets are activated in PRP and not observed when using washed isolated platelets (104, 105). Because the platelet FcγRIIa functional polymorphism was observed only in PRP and not in washed platelets, we suggested that other important plasma factor(s) and mechanisms are involved in platelet FcγRIIa functional polymorphism (105).

The risk of developing thrombosis in VITT may depend on platelet FcγRIIa polymorphism; this has been reported to be a risk factor for HIT (62, 63). Further, *in vitro* studies and investigations may be needed to determine the role of platelet FcγRIIa polymorphism in VITT by comparing the platelet aggregation elicited *in vitro* by purified VITT IgG anti-PF4 in PRP vs. washed platelets, including platelets from different apparently healthy subjects defined as "responders", "intermediate-responders" or "non-responders" to mouse activating platelet antibodies; such as the PL-249 monoclonal antibody.

## VITT incidence rates: COVID-19 Oxford/AstraZeneca and Johnson & Johnson/Janssen vaccines and their relation to VITT

VITT is very rare, however, it can be life threatening, especially if the diagnosis and treatment are delayed. It remains highly relevant in countries that afforded and can only afford adenoviral vector-based vaccines for COVID-19 vaccination campaigns. The vaccination campaign, combined with an educational program informing the public and especially physicians about VITT symptoms and appropriate treatment, allowed a reduction in mortality from 50% to 5% - 6% in Australia (118). In **Table 4**, we focus on the COVID-19 Oxford/AstraZeneca and Johnson & Johnson/Janssen vaccines, used in the Western world with most reported VITT cases. The VITT incidence caused by COVID-19 Oxford/AstraZeneca and Johnson & Johnson/Janssen vaccines varies by the reporting country.

## Worldwide population and demographics reaction to COVID-19 vaccines

Vaccination is critical for controlling the COVID-19 pandemic. As of 30 June 2022, 66.4% of the world's population had received at least one dose of a COVID-19 vaccine, however only 17.4% of people in low-income countries had received a first dose, underlying unequal access, availability and delivery (120–122). Despite the reductions in COVID-19 disease severity, hospitalizations and deaths, since the introduction of multiple safe and effective

TABLE 4 Incidence of VITT in relation to COVID-19 Oxford/AstraZeneca and Johnson & Johnson/Janssen vaccines (119).

| Manufacturer | Oxford/AstraZeneca | Johnson & Johnson/Janssen |
|---|---|---|
| Vaccine efficacy | 63% | 67% |
| VITT Incidence, as of Q1 2022 | 1/64,000–1/125,000 | 1/310,000–1/200,000 |
| VITT mortality rate, as of Q1 2022 | 18% | 15% |
| Total number of vaccines administered in the USA or EU (in millions) | 67 | 37 |
| Suspension | ■ In late February 2021, reports of thrombosis with thrombocytopenia occurring after vaccination led to the temporary suspension of its use in multiple European countries.<br>■ By 10 March 2021, 30 cases of thromboembolic events in response to five million vaccinations were reported by the European Medicines Agency. | ■ The U.S. Food and Drug Administration (FDA) and Centers for Disease Control and Prevention (CDC) suggested pausing the administration of Johnson & Johnson/Janssen in April 2021 due to six reports of TTS after 6.8 million doses were administered.<br>■ In December 2021, the CDC recommended against the use of the vaccine.<br>■ In May 2022, the FDA officially limited the authorized use of Johnson & Johnson/Janssen to adults for whom other authorized COVID-19 vaccines were not accessible or clinically appropriate, or for adults who declined other authorized COVID-19 vaccine options. |

COVID-19 vaccines, vaccine hesitancy and refusal remains substantial, caused in part by misinformation, lower education, mistrust in science and governments, and elusive broad public support (123). The study of COVID-19 vaccine hesitancy among 23,000 respondents in 23 countries during 2022, representing almost 60% of the world's population, showed willingness to accept vaccination at 79.1%, however one out of eight (12.1%) vaccinated respondents are hesitant about booster doses (123). Twice-yearly COVID-19 booster vaccinations are recommended in some countries based on eligibility and availability. The same factors that influence hesitancy to accept an initial COVID-19 dose also drive booster hesitancy (124). COVID-19 vaccine hesitancy persists for vaccine boosters, which may present a serious challenge to anticipated routine COVID-19 immunization programs. Worldwide, policymakers and public health officials must address COVID-19 vaccine hesitancy and resistance as a component of their overall prevention and vaccination strategy.

## Discussion & conclusion

Vaccines are vital and invaluable to control pandemics and to build up herd immunity. They have considerably reduced the incidence of childhood illnesses, notably measles, mumps, and rubella, and have facilitated the eradication of many infectious diseases, such as poliomyelitis, diphtheria, and smallpox (9). From the onset of COVID-19 pandemic to November 2021, more than 771 million cases and 6,960,7835 million deaths were documented worldwide. Vaccination for prevention against COVID-19 is crucial in containing the spread of SARS-CoV-2 and controlling this pandemic. For the Covid-19 pandemic, the use of diverse technologies allowed the development and production of highly effective vaccines, all produced with unprecedented timelines. The two most common COVID-19 vaccine platforms currently in use are the messenger RNA (mRNA) and adenovirus vector vaccines. The development of antibodies directed against the SARS-CoV-2 spike protein is the strategy chosen by most vaccine developers.

The overwhelming inflammatory response in patients with SARS-CoV-2 infection can lead to VITT associated with a hypercoagulable state, thrombosis, large vessel thrombosis, and, ultimately, death. VITT's overwhelming inflammatory bodily reaction to COVID-19 vaccination was observed in a small minority of vaccine recipients. The risk of VITT associated with thrombosis and mortality should be considered considerably subordinate to the relative risk of loss of life and morbidity attributable to the SARS-CoV-2 infection. VITT recognition raised concerns regarding the safety of COVID-19 vaccines and led to the reconsideration of vaccination strategies in many countries. Prior to vaccination, individuals should be educated about the clinical symptoms associated with VITT syndrome and should be advised to seek urgent medical treatment. VITT appears to be similar to HIT-, SK-, and APSC - induced platelet activation through platelet Fc$\gamma$RIIa, all showed strong, dose-dependent respective antibodies-mediated platelet activation, which is completely inhibited by IV.3, an Fc$\gamma$RIIa-blocking monoclonal antibody. In the event of major thrombotic events 4–30 days after COVID-19 vaccination, VITT should be considered; its treatment consists of therapeutic anticoagulation with nonheparin anticoagulants, and the infusion of high-dose IVIG to neutralize the anti - PF4 antibody-Fc$\gamma$RIIa binding. Routine platelet transfusions, aspirin, and warfarin should be avoided because of the possibility of worsening thrombosis and magnifying bleeding risk. There are no well-known risk factors for VITT with CVST post vaccination.

VITT was observed in both males and females across a wide age group, thus, there may not be a gender or age restriction in regard to suspected VITT. Therapeutic anticoagulation with nonheparin anticoagulants is the primary treatment for VITT with or without CVST. Given the concern of VITT, healthcare providers should be familiar with the VITT clinical presentations, pathophysiology, diagnostic criteria, and management consideration. Furthermore, they should maintain a high index of suspicion in patients presenting with symptoms on laboratory abnormalities suggestive of VITT as early recognition and management of this syndrome can prevent catastrophic complications. With increased VITT awareness and recognition by providers and the public alike, future cases should help refine VITT clinical understanding and improve its clinical outcomes. The better understanding of the mechanisms behind the VITT thrombotic complications is needed to aid in better disease management and improve the prognosis. Vigilance should be maintained regarding large-scale injections of vaccines, which contain adenoviruses and genetic material from the SARS-CoV-2 spike protein.

In immune complex disorders, platelets interact with IgG-containing immune complex through platelet Fc$\gamma$RIIa. The similarities of the mechanisms of VITT-, HIT-, SK, and APSAC-induced platelet activation could be relevant for a better understanding of immune diseases associated with abnormal hemostasis and thrombosis in general, and to VITT in particular. The exact VITT pathogenic mechanism is yet to be fully elucidated, further, *in vitro* as well as *ex vivo* experiments, investigations and specific lines of inquiry are warranted to reveal the true relationship that lies between COVID-19 vaccinations with VITT, and VITT possible risk factors in order to implement risk minimization strategies.

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MA: Writing – original draft, Writing – review & editing. DY: Writing – original draft, Writing – review & editing. SF: Writing – review & editing.

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## Conflicts of interest

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## Publisher's note



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# Corrigendum: FcγRIIa—dependent platelet activation identified in COVID-19 vaccine-induced immune thrombotic thrombocytopenia-, heparininduced thrombocytopenia, streptokinase- and anisoylated plasminogen-streptokinase activator complex-induced platelet activation

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platelet activation, COVID-19, vaccine induced immune thrombotic thrombocytopenia, vaccination, FcγRIIa

A Corrigendum on
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In the published article, there was an error in **Figure 1** as published. The incorrect image for **Figure 1** was used. The corrected figure appears below.

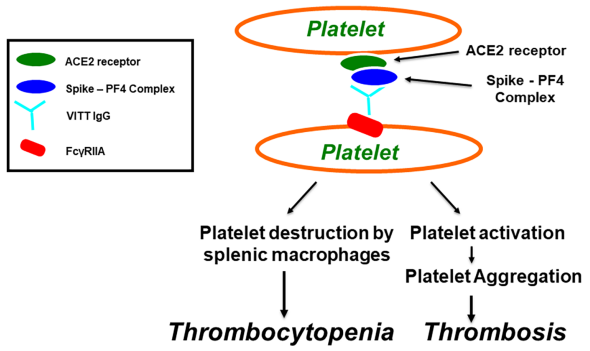


FIGURE 1
Vaccine-induced immune thrombotic thrombocytopenia (VITT). Antibodies induce platelet aggregation through FcγRIIa. SARS-CoV-2 virions, or its spike protein, produced after COVID-19 vaccination, bind to platelets via ACE2 receptor, leading to activation of platelets and the secretion of platelet factor 4 (PF4). PF4 then biochemically associates with the spike protein, forming PF4-Spike complexes that stimulate VITT anti-PF4 antibody production. VITT pathological IgG antibodies induce platelet aggregation through platelet FcγRIIa and thrombocytopenia through platelet destruction by splenic macrophages.

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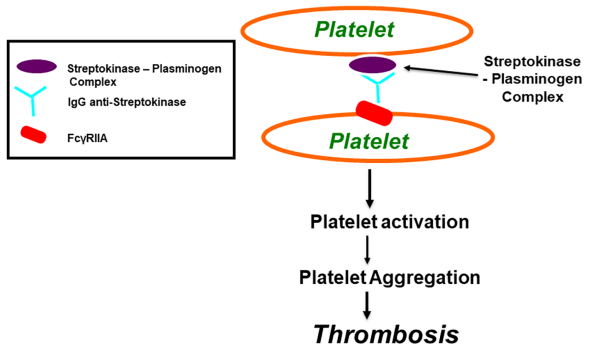


FIGURE 3
Streptokinase (SK) and Anisoylated Plasminogen-Streptokinase Activator Complex (APSAC) induced platelet aggregation through FcγRIIa. Both Streptokinase and APSAC modify in vitro platelet aggregation by two mechanisms; reduced aggregation due to fibrinogenolysis, and enhanced aggregation via an immunological reaction. The reduced aggregation by SK (or APSAC) is mediated by plasmin generation and the fibrinogen degradation product, fragment E. As shown in this figure, SK (or APSAC) may also trigger platelet aggregation by a mechanism involving specific IgG anti-SK. Both SK- (or APSAC) induced platelet aggregation and SK- (or APSAC) enhanced ADP-induced platelet aggregation require the interaction of the Fc domain of the anti-SK antibodies with the platelet FcγRIIA.

The authors apologize for these errors and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

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# New insights of platelet endocytosis and its implication for platelet function

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Endocytosis constitutes a cellular process in which cells selectively encapsulate surface substances into endocytic vesicles, also known as endosomes, thereby modulating their interaction with the environment. Platelets, as pivotal hematologic elements, play a crucial role not only in regulating coagulation and thrombus formation but also in facilitating tumor invasion and metastasis. Functioning as critical components in the circulatory system, platelets can internalize various endosomal compartments, such as surface receptors, extracellular proteins, small molecules, and pathogens, from the extracellular environment through diverse endocytic pathways, including pinocytosis, phagocytosis, and receptor-mediated endocytosis. We summarize recent advancements in platelet endocytosis, encompassing the catalog of cargoes, regulatory mechanisms, and internal trafficking routes. Furthermore, we describe the influence of endocytosis on platelet regulatory functions and related physiological and pathological processes, aiming to offer foundational insights for future research into platelet endocytosis.

KEYWORDS

platelet, endocytosis, cargo contents, thrombosis, phagocytosis

## 1 Introduction

Platelets are small, anuclear blood cells that derived from bone marrow megakaryocytes (MKs) or distant organs such as lung or liver, and play an essential role in hemostasis and thrombogenesis (1–3). At sites of vascular injury, platelets are instantly captured from blood stream, adhering to the subendothelial matrix at exposed endothelial surfaces to form thrombi (4). In addition to hemostatic function, platelets are instrumental in diverse physiological and pathological processes, including inflammation and immune response, antibacterial host defense, liver regeneration, tumorigenesis and metastasis (5). It has been proved that platelet activation is accompanied by the occurrence of exocytosis, during which platelet granules release their procoagulant contents to extracellular milieu. Furthermore, research on platelets has highlighted the critical role of endocytosis in their biological functions. Platelet endocytosis conception was first proposed more than 30 years ago, while the underlying mechanism of platelets endocytic pathways are relatively limited in definition (6).

Endocytosis usually refers to as the uptake of extracellular cargoes and the transport of cell surface receptor through various membrane-binding chambers named endosomes (7). With the plasma membrane invagination, extracellular substances are wrapped into cells and delivered to early endosomes. After that, early endosomes transmit the cargoes to

distinct intracellular locations, such as degrading in the late endosomes, recycling to the platelet membrane, or secretion (8).

The phenomenon of platelet endocytosis has been acknowledged since the 1980s when the existence of active internal systems in platelets was first identified (7, 9, 10). Technological advancements have enabled the observation of platelets internalizing small particles (such as fibrinogen, IgG, and albumin) into membranous structures through a phagocytosis-like mechanism, as revealed by transmission electron microscopy (TEM) (11–15). Further research has unequivocally established the substantial role of endocytosis in platelet physiology. Beyond influencing the transport of granules and cargoes, platelet endocytosis is implicated in hemostasis and various pathophysiological processes (16). In this review, we aim to provide a concise summary of platelet endocytosis, covering the transported contents, molecular mechanisms, transport pathways, and its impact on platelet-associated physiological and pathological phenomena. This overview is intended to offer a systematic introduction and to foster novel insights for subsequent investigations into platelet endocytosis.

# 2 Cargoes uptake by platelets endocytosis

## 2.1 Plasma contents ingested by platelet granules

Platelets release more than 300 bioactive substances including multiple proteins, peptides and small molecules (nucleotides, serotonin, $Ca^{2+}$, etc.), which mediate the diverse functions of platelets (17, 18). The bioactive substances are mainly packed in three kinds of intracellular secretory granules in platelet: α-granules, dense granules and lysosomes (19–21).

Platelet α-granules contain more than 280 proteins including coagulation factors, which are of fundamental importance in hemostasis (22). Some of these proteins are platelet-specific, for example, β3-thromboglobulin. Some counterparts are from plasma, including albumin, fibrinogen, fibronectin, von Willebrand factor (vWF), etc. Originally, it was believed that proteins in granules originated exclusively from *de novo* synthesis during megakaryocyte (MK) development. However, subsequent studies have revealed that circulating plasma proteins can be internalized into MKs or platelet granules via endocytic pathways, and then returned to the plasma (14, 23, 24).

Factor I (fibrinogen) is the primary adhesion protein secreted by platelets, accounting for 10% of α-granule proteins (12). Harrison provide the first clear evidence for *in vivo* endocytic uptake of fibrinogen from plasma by human platelet α-granules (23). Fibrinogen in platelets is not synthesized by MKs (25), and can only be obtained from plasma by endocytosis (12, 23, 26). It has been shown that factor III (Tissue Factor, TF) can be internalized by platelets into the open canalicular system (OCS) channels, followed by its accumulation in the platelet cytoplasm and, occasionally, within α-granules (24, 27, 28). However, the storage of TF in α-granule of unstimulated platelets is a subject of ongoing debate, since there is evidence of TF-mRNA in platelets (29). Factor V, necessary for the optimal conversion of prothrombin to thrombin, is present in significant concentrations in blood platelets and localized within the α-granules of unstimulated platelets. Research has localized factor V within the α-granules of unstimulated platelets (30, 31). Different from fibrinogen, factor V was endocytosed by MKs, but not by platelets (32). In addition to these coagulation factors, vascular endothelial growth factor (VEGF) (33), intravenously injected albumin, immunoglobulin G (14), insulin-like growth factor (IGF) I and IGF-binding protein-3 are also known to be ingested by MKs and incorporated into platelet α-granules (34), highlighting the complex and selective nature of protein uptake by platelets.

Dense granules within platelets contain small molecular substances, categorically divided into four primary types: 1. Nucleotides, including Adenosine Diphosphate (ADP) and Adenosine Triphosphate (ATP); 2. Amines, exemplified by serotonin; 3. Cations, such as calcium ions; and 4. Phosphates, notably pyrophosphate and polyphosphates (35). 5-hydroxytryptamine (5-HT), also known as serotonin, is a neurotransmitter. Platelets do not synthesize 5-hydroxytryptamine (5-HT), commonly known as serotonin, but rather acquire it from the bloodstream via the serotonin transporter (SERT), subsequently storing it in their dense granules (36). Serving as the primary carrier of serotonin in the blood, platelets play a pivotal role. The release of serotonin from platelets is crucial in enhancing the wound healing process in various organs (37).

## 2.2 Endocytic recycling surface receptors in platelets

A variety of receptors are expressed on the surface of platelets, enabling the recognition of extracellular ligands, matrix components, and receptors on other cells. Upon platelet activation, extracellular ligands bind to these surface receptors, providing a physical anchor and triggering the intracellular signaling events (38). Moreover, stimulation by agonist such as thrombin and ADP causes migration of platelet receptors from the interior to the cell surface and re-distribution (39–41). The dynamic turnover of endocytic and exocytic receptor trafficking contributes to cell invasion, metastasis and cytokinesis (42).

### 2.2.1 Integrin αIIbβ3

Integrins are a family of adhesive receptors whose functional regulation depends not only on the conformational change but also via integrin trafficking (43). Recycling of integrins can rapidly deliver integrins back to the plasma membrane, thus providing the cell with a constant fresh reservoir for new adhesions (38).

Integrin αIIbβ3, uniquely expressed by platelets, serves as a key adhesion molecule pivotal in thrombosis control (44). Previous study has shown that there is a dynamic pool of αIIbβ3 in human platelets that is obviously internalized through the

endocytic vesicles formation. Intriguingly, upon thrombin stimulation, these vesicles are capable of migrating to the cell surface (45). Consequently, the internalization process of integrin αIIbβ3 in platelets may constitute a regulatory mechanism for adhesion receptors (46). Huang et al. have elucidated the crucial role of αIIbβ3 endocytosis in platelets and proved that changes of integrin αIIbβ3 trafficking may influence platelet functions, particularly in terms of spreading and clot retraction (47).

### 2.2.2 $P2Y_1$ and $P2Y_{12}$ purinergic receptors

ADP is of great significance for platelets activation. Two types of G protein-coupled receptors (GPCRs) expressed on platelet surface, $P2Y_1$ and $P2Y_{12}$, are activated by ADP (48, 49). Upon stimulation, $P2Y_1$ is coupled to $G_q$ and PLCβ, while $P2Y_{12}$ is negatively coupled to adenylyl cyclase by $G_i$) thus promoting complete platelet aggregation. ADP induced platelet activation is usually initiated by $P2Y_1$ and amplified by $P2Y_{12}$ receptor (50).

Mundell and colleagues have showed that both $P2Y_1$ and $P2Y_{12}$ could be internalized rapidly in human platelets by a radioligand binding approach (51). Their findings also revealed that inhibitors of protein kinase C (PKC) significantly diminish ADP-induced internalization of the $P2Y_1$ receptor, while having no substantial effect on the $P2Y_{12}$ receptor (51). With further research, they demonstrated that the endocytosis of $P2Y_1$ and $P2Y_{12}$ receptors relies on dynamin, which is indispensable for resensitization of responses (50). An intact putative postsynaptic density 95/disc large/zonula occludens-1 (PDZ)-binding motif is essential for proper internalization and subsequent recycling of the $P2Y_{12}$ purinoceptor in human platelets (52). Moreover, The PDZ-binding protein $Na^+/H^+$ exchanger regulatory factor (NHERF) binds to the $P2Y_{12}$ receptor to promote agonist-dependent internalization. In this process, arrestin can act as an adaptor to scaffold NHERF1 to these GPCRs thus motivating receptor internalization (53). Arf6 also participate in P2Y receptors internalization which will be discussed in the following text.

### 2.2.3 CLEC-2

The hemi-immunoreceptor tyrosine-based activation motif–containing C-type lectinlike receptor 2 (CLEC-2) is a transmembrane protein that highly expressed in platelets and plays crucial roles in platelet activation (54). Besides contribution to thrombosis, platelet CLEC-2 is also involved in the regulation of tumor metastasis, blood-lymphatic vascular development, and inflammatory bleeding (55). CLEC2 regulates platelet activation mainly by influencing a Src- and spleen tyrosine kinase (Syk)-dependent tyrosine phosphorylation cascades.

Lorenz et al. have showed that by injecting INU1 (a monoclonal anti-body) *in vivo* can specifically remove CLEC-2 from platelets and MKs. Nonetheless, this intervention was observed to cause a marked decrease in CLEC-2 levels, leading to thrombocytopenia, which poses a challenge for its therapeutic use (55). The research indicated that the reduction in CLEC-2 due to INU1 is primarily a consequence of receptor internalization within the platelets, governed by the activity of Src-family kinases. Intriguingly, while the Syk kinase is not crucial for the decrease in CLEC-2 triggered by INU1, it is vital for the onset of thrombocytopenia. Thus, selectively inhibiting platelet Syk can counteract the thrombocytopenia caused by INU1, without interfering with the process of CLEC-2 internalization (55).

### 2.2.4 GPIb

Platelet membrane glycoprotein (GP) Ib is a receptor for vWF. Binding of vWF to GPIb is important in the initiation of the process of platelet adhesion to damaged blood vessel walls (56). Previous studies have observed the presence of GPIb pools inside the platelets (57, 58). Redistribution of platelet surface and internal cisterna of GPIb was observed within platelets, during which endocytosis of GPIb receptor may occur (59). Jones et al. have established that platelet endothelial cell adhesion molecule-1 (PECAM-1) could regulate platelet GPIb internalization through dual AKT/protein kinase B/glycogen synthase kinase-3/dynamin-dependent and αIIbβ3-dependent pathways, so as to inhibit thrombin and vWF induced platelet activation (60).

### 2.2.5 Mpl/TPOR

Thrombopoietin (TPO), together with its receptor, Mpl, can facilitate megakaryocyte differentiation, promote survival and proliferation of hematopoietic stem cells and progenitor cells (61). Receptor-mediated internalization appears to be the primary regulating way of plasma TPO level. Mpl could be internalized in human platelets, without recycling to the cell surface (62). Recent research suggested that endocytosis of Mpl was restricted in Dynamin 2 (DNM2) knock out platelets, which also resulted in phosphorylation of the tyrosine kinase JAK2 and increased TPO levels (63). In conclusion, in MKs, DNM2-dependent endocytosis plays important roles during its development.

## 2.3 Virus and bacteria

In addition to the hemostatic function, platelets also act as innate immune cells involved in inflammation and immune response *in vivo* (64). Platelets express some pathogen recognition receptors, e.g., complement receptors, αIIbβ3, FcγRIIa, CLEC-2, GPIbα and dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), which enable platelets to detect and interact with bacterial and viral particles (65). Several studies have shown that platelets can bind and phagocytose infectious microorganisms (66–69). Platelets could promote the transport of infection *in vivo* by taking up viruses, and they also may help the host organism to resist infection. It is proposed that herpes virus with larger DNA, such as herpes simplex virus (HSV)-1, only bind to platelets without internalization (70–72). In contrast, some smaller RNA viruses, are endocytosed by platelets, as shown in Figure 1 and described below.

### 2.3.1 Human immunodeficiency virus type I (HIV1)

HIV1 is a single positive-stranded RNA virus belonging to the *Retroviridae* family, genus *Lentivirus*. In 1990, Zucker-Franklin D

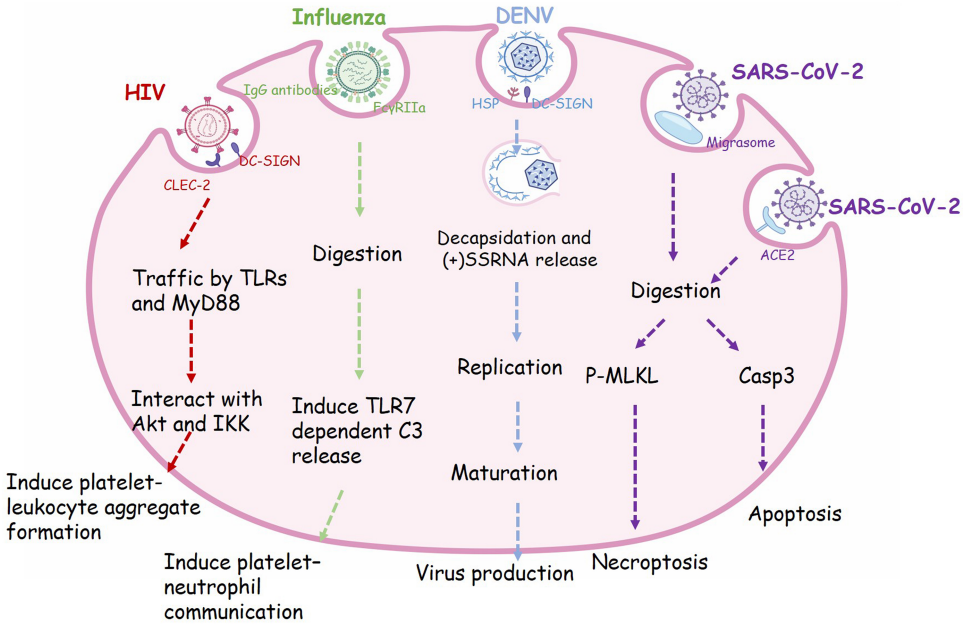


FIGURE 1
The phagocytic receptor, trafficking routes and regulatory functions of viral endocytosis in platelets. Many receptors expressed on the surface of platelets are involved in the process of virus phagocytosis. HIV enter platelets by binding to dendritic cell-specific ICAM-grabbing non-integrin (DC-SIGN) and C-type lectin-like receptor 2 (CLEC-2), then traffic via Toll like receptors (TLRs) and Myeloid Differentiation primary response protein 88 (MyD88) and interact with Akt and (IKK), finally induce granule release and platelet-leukocyte aggregate formation. Influenza viruses are recognized by IgG-specific antibodies and form an immune complex, which in turn is recognized by FcγRIIa, resulting in the internalization of this complex. Engulfment of influenza will activate platelet TLR7 and initiate granule release of complement C3 (C3), thus lead to NETosis. Dengue virus (DENV) binds to platelets via DC-SIGN and heparin sulfate proteoglycans (HSP). The viral particle go through decapsidation, ssRNA replication, maturation, and virus production. SARS-CoV-2 virions can be taken up into platelets through Angiotensin converting enzyme-2 (ACE2) or by attaching to microparticles. Phagocytosis of SARS-CoV-2 will induce apoptosis mediated by caspase 3 (Caps-3) and necroptosis mediated by phosphorylation of mixed lineage kinase domain-like pseudokinase (MLKL), finally lead to programmed cell death in platelets.

et al. first observed internalization of HIV1 and other retroviruses in MKs and platelets (73). In vitro experiments, Flaujac and colleagues incubated platelets with HIV viruses for 30 min. They found that HIV was completely internalized, maintaining the integrity of its ultrastructure. Characteristic endocytic vacuoles containing HIV particles were found near the plasma membrane (66). In addition, the endocytosis of HIV also occurred *in vivo* after test in platelets from clinical patients with AIDS (67). HIV-1 can be completely encased in platelets, transported through the body by circulating platelets and protected from attack by the host immune system (64). Platelets that internalize viruses undergo activation and exhibit P-selectin expression on their membrane. Subsequently, these P-selectin positive platelets are recognized by macrophages and efficiently cleared from the circulation (66). The thrombocytopenia complication after HIV infection may be partly caused by this defense mechanism (66).

### 2.3.2 Influenza virus

Influenza is a respiratory virus, and infection of it will lead to barrier damage of epithelial-endothelial, allowing the entrance of virus into blood (68). In 1959, the incorporating of influenza virus into platelets was first observed by electron microscopy (74). Koupenova et al. proposed that incubation of platelets with H1N1 strain (WSN/33) leads to internalization of the viral particles by platelets, which is morphologically similar to phagocytosis, and the internalized virus are rapidly digested (75). As a matter of fact, almost all influenza A strains (H1N1, H5N1, and H3N2, etc.) can bind to platelets, albeit with varying affinities (H5N1 > H1N1 > H3N2) (76). Further studies are required to confirm whether and how these viruses are endocytosed into platelets. Overall, platelets may be a line of intravascular defense against influenza, as well as other immunity cells.

### 2.3.3 Dengue virus (DENV)

Dengue fever is a mosquito-borne viral disease mainly found in tropical and subtropical regions. Infection of dengue virus in patients will cause capillary leakage, bleeding, and organ compromise, and in severe cases, it can lead to shock or even death (77). Platelets are crucial in the pathogenesis of DENV, as the infection is often accompanied by thrombocytopenia (78). Therefore absolute platelet count is used as lab indication to examine the disease progression of DENV (77). Researches have established that dengue induces platelet activation, mitochondrial dysfunction and apoptosis (79, 80). It is also suggest that DC-SIGN may participate as a key receptor in DENV mediated platelet activation (79). Ayo Y. Simon et al. found that DENV in blood can utilize DC-SIGN and heparan sulfate proteoglycan as

primary receptors to bind to platelets directly. Platelets could endocytose this virus, replicate and produce infectious DENV. Usually platelets serve as an important line of defense against blood-borne viruses. However dengue appears to take advantage of this feature by getting inside the platelets and replicating further, increasing the survival chances of virus (78). The mechanism of DENV taking up by platelets remains unclear and requires further study, which can serve as new target for the DENV treatment.

### 2.3.4 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

SARS-CoV-2 is a new type of positive-sense single-stranded RNA beta coronavirus which induced coronavirus disease 2019 (COVID-19) pandemic (81). Infection of SARS-CoV-2 not only causes acute respiratory distress syndrome (ARDS), but also evokes a series of thrombotic complications, and leads to organ failure and even mortality (81, 82). These clinical complications of cardiovascular disease successfully raise concern about relationship between platelet activation and SARS-CoV-2.

Platelets in patients with COVID-19 exhibit hyper-activity, evidenced by elevated integrin αIIbβ3 activation and P-selectin expression (69). Observation of platelets from patients with severe SARS-CoV-2 infection showed viruses could enter platelets surface and intracellular platelets, suggesting that SARS-CoV-2 might be ingested by platelets. The mRNA traces of SARS-CoV-2 were detected in the isolated platelets, and virions could be observed by electron microscopy within platelets sections (69, 83, 84).

Milka Koupenova et al. found that the internalization and digestion of the SARS-CoV-2, changes in platelet morphology, and release of extracellular vesicles can be observed after co-incubating platelets with purified virions (85). Angiotensin converting enzyme-2 (ACE2) is recognized as the primary receptor for entry of SARS-CoV-2 into host cells (69). Koupenova and colleagues propose that both ACE2 dependent and independent endocytosis of SARS-CoV-2 occur in platelets. They also observed a class of microparticle related to platelets which might be migrasome (85). During cell migration, migrasome functions as local chemo-attractants and mediating release of cytoplasmic contents (86). Platelets can take up SARS-CoV-2 virions attached to microparticles (85). Following the internalization of SARS-CoV-2 by platelets, researchers have observed a colocalization of the virus with phospho-MLKL (mixed lineage kinase domain-like pseudokinase) and caspase 3. This interaction leads to a rapid onset of platelet cell death, during which the virus seems to fragment, inhibiting its further replication and spread. This suggests that platelets may play a protective role in the immune response against the virus. However, with the death of platelets, their internal active substances are also released, resulting in pro-thrombotic complications in COVID-19 patients.

### 2.3.5 Bacteria

During bacterial infection and sepsis, direct and indirect interactions through a wide range of cellular mechanisms between platelets and bacteria may contribute to thrombocytopenia (87). In the microvasculature, bacterial cells could activate platelets, and cause thrombosis (88). Several studies have shown that *Staphylococcus aureus* (*S. aureus*) can induce aggregation and ATP release of platelets, and it is occasionally observed that *S. aureus* can be internalized by individual platelets (89–91). Moreover, platelet activation could increase the internalization of bacteria. During endocytosis, platelets appeared to elongate pseudopods that surrounded the *S. aureus* and completely enclosed the particles in a vacuole, in a manner similar to phagocytes engulfing bacteria. In the final stage of endocytosis, *S. aureus* seems to be closely contact with α-granule secretory fibrinogen within platelets (67).

In addition to *S. aureus* (a gram-positive bacterium), Porphyromonas gingivalis (*P. gingivalis*, a gram-negative bacterium) have also been reported to interact with platelets and cause aggregation of platelets (92). Xiangfeng Li et al. found that during bacterial induced platelet activation, *P. gingivalis* was engulfed in the OCS, and then internalized into the platelet cytoplasm. Streptococci and other bacteria have also been proved to interplay with platelets (88), however the detailed process of bacterial endocytosis into platelets has not been discussed and awaits further study.

## 2.4 Drugs endocytosis by platelet as smart drug delivery system

The controlled and efficient delivery system of drugs is essential for the clinical treatment of disease. An intelligent and controllable drug delivery system that effectively targets specific organs or tissues should maintain its bioavailability and minimize side effects (93). Platelets are endowed with the capacities of storage, transport, and release, making them potential carries for drug delivery systems (94). Moreover, when the drug is loaded in platelets, it could be protected from the clearance of immune system, and thus may allow a prolonged circulation time in blood.

Previous study has shown a strong link between platelets and cancer (95). Tumor cells can induce platelet adhesion, aggregation and release. During this process, the drugs loaded in platelets are released along with particulate components to the tumor site (96). The endocytosis properties of platelets can be used to load drugs, providing a promising way for guiding drugs to the blood circulatory system and complex tumor microenvironment.

### 2.4.1 Doxorubicin

Doxorubicin (DOX) is a chemo-therapeutic drug used in the treatment of lymphoma, but it has many side effects such as short biological life time and cardiotoxicity due to non-specific bio-distribution (97). The utilization of DOX-loaded platelets as a drug delivery system, achieved by incubating doxorubicin (DOX) with a platelet suspension at 37°C, has been investigated in multiple studies, particularly focusing on lymphoma treatment (93, 96). By this means, the researchers successfully prolonged DOX retention time compared to synthetic drug delivery systems (96, 98). Dox-loaded platelets facilitate the gathering of

intracellular drug through platelet aggregation caused by tumor cells and release DOX in a pH-controlled manner. This method could reduce the side reaction of chemotherapy drugs to the patient and improve the therapeutic effect in clinical treatment of lymphoma (99). However, the precise mechanism underlying platelets' internalization of doxorubicin (DOX) remains unclear, as it is not explicitly detailed in these studies. The process of DOX encapsulation within platelets likely involves intricate biochemical pathways, possibly engaging the open canalicular system (OCS) of the platelets (96, 98). This area warrants further investigation to elucidate the exact mechanisms involved.

### 2.4.2 Monoclonal antibody bevacizumab

Platelets are the main source of VEGF in blood. Platelets activation leads to release of growth factors from α-granule, including VEGF, which promotes angiogenesis (100). Bevacizumab is a monoclonal antibody that blocks VEGF and has been used in the treatment of cancer disease. Previous study demonstrated that platelets can take up bevacizumab (101). Immunofluorescence microscopy displayed that bevacizumab phagocytosed in platelets colocalized with P-selectin. They found that platelets from patients receiving bevacizumab treatment can endocytose bevacizumab (101). Platelet endocytosis is still worthy of further study in the direction of drug delivery with platelets as carriers, which is of great significance for targeted treatment of tumors and cardiovascular diseases.

## 3 Regulators of platelet endocytic machinery

Platelet endocytosis has been long recognized, however, the molecular machinery, remain understudied. Up to now, several molecules have been proved to be involved in the regulation of platelet endocytic mechanism (Figure 2). According to existing studies, multiple pathways of endocytosis could be broadly classified as clathrin-dependent and clathrin-independent internalization (102).

### 3.1 Clathrin dependent endocytosis

Clathrin is a protein which can form a polymer on the downside of the coated pit. Endocytosis mediated by clathrin

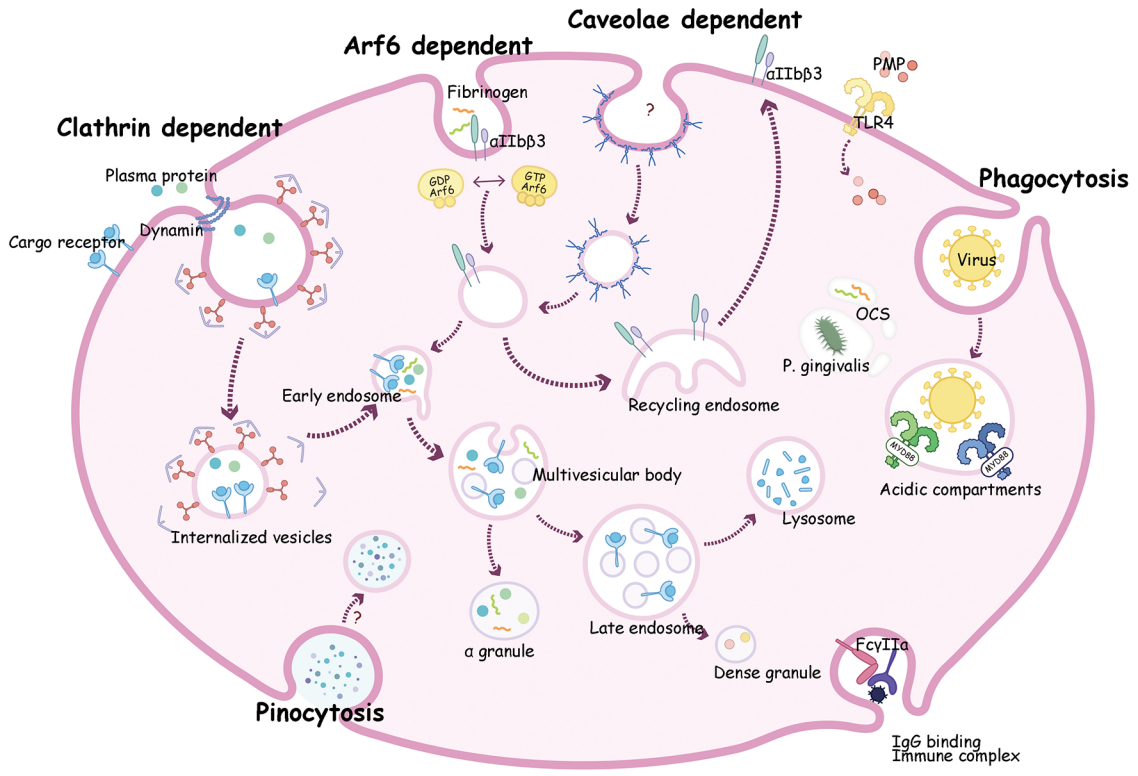


FIGURE 2
A composite description for endocytosis mechanism and transportation pathways in platelets. Clathrin dependent endocytosis can transport plasma proteins such as vWF and fibrinogen (Fg) with or without receptor into platelets. These proteins go through early endosome then be sorted to multivesicular bodies (MVB) and ultimately into α-granules for storage. Alternatively, cargoes can move into late endosomes then transit into dense granules or to lysosomes where it may be degraded or stored. Receptors such as $P2Y_{12}$ and αIIbβ3 are taken up by Arf6, then circulate to recycling endosome, and finally return to the plasma membrane. Virions may enter platelets by phagocytosis, then bind to endosomal TLRs, and activate a myeloid differentiation primary response protein 88 (MyD88)- based signaling cascade, and finally been digested. Proteins like fibrinogen and bacteria such as *P. gingivalis* can be internalized into the open canalicular system (OCS) channels.

usually depends on clathrin-coated vesicles (103). In platelets, clathrin-coated membranes were first noticed in the 1980s (9). The formation of clathrin encapsulated vesicles requires the following five steps: initial stage, selection of transport goods, coat assembly, scission and coating removal (103). In this process, extracellular molecules integrate with the ectodomain of receptors, then accumulate in coated pits on the cell membrane (16). The pits are further transformed into clathrin-coated vesicles with short-life (104). Subsequently, the coat of vesicles falls off, and the remaining portion fuses with endosomes and is transport along the endocytosis pathway (42, 105). By utilizing a novel inhibitor of clathrin named pitstop 2, Wen Gao et al. suggest that integrin αIIbβ3 trafficking mediated by clathrin controls spreading during platelet activation (42).

### 3.1.1 Dynamin

Dynamins (DNMs) are highly conserved mechanochemical GTPases regulate endocytosis and vesicle transport (63, 106). After the formation of clathrin-coated vesicles, DNM is recruited to promote the vesicle scission from the neck (42). There are three members of DNM family: DNM1, DNM2, and DNM3. Human platelets express all DNMs, whereas mouse platelets mainly express DNM2 (107, 108).

Platelets contain low levels DNM1, as it mainly expressed in brain (109). Previous studies have suggested DNM3 participate in MK maturation and platelet formation (110, 111). DNM2 mutations are associated with thrombocytopenia and hematopoietic diseases (112, 113). Bender et al. found that deletion of DNM2 in platelets significantly suppressed the endocytosis of the TPO receptor, Mpl. In conclusion, DNM2-dependent endocytosis is essential for megakaryopoiesis, thrombopoiesis, and bone marrow homeostasis (63).

### 3.1.2 Disabled-2 (Dab2)

Disabled-2 (Dab2) acts as an adaptor protein in clathrin-mediated endocytosis, and participates in transport of many receptors and intracellular signaling (114). Dab2 has two alternative splicing isoforms p82 and p59 (115). P82-Dab2 is mainly distributed in the cytosol and on α-granules of human platelets, which regulates fibrinogen binding and platelet aggregation (116). P59-Dab2 is abundant in mouse platelets, and it is essential for fibrinogen ingestion, RhoA-ROCK activation, ATP secretion, and integrin αIIbβ3-mediated signaling (117). Knock out of Dab2 specifically in mice platelets showed a bleeding tendency and impaired thrombosis. It has been proved that Dab2 shows strong interaction with $G\alpha_{12}/_{13}$-mediated thrombin signaling in hemostasis (118). Dab2 is a key player in the endocytosis of platelet Integrin-β3. Cheng-han Yu and colleagues have reported that the turnover of Integrin-β3 in platelets is modulated in a manner dependent on cell-matrix force interactions. Under the influence of traction forces, talin directly associates with Integrin-β3, leading to the formation and maturation of focal adhesions. Conversely, the absence of physical forces on RGD-glass triggers the recruitment of Dab2 and clathrin, culminating in the internalization of activated integrins (119).

### 3.1.3 IFN-induced transmembrane protein 3 (IFITM3)

IFITM3, an type I interferon (IFN)-responsive gene that plays critical roles in restriction of viral replication (120). During infection of dengue and SARS-CoV-2 virus, expression of IFITM3 significantly increased in MKs and platelets, thus limiting the entry and replication of virus in the cytoplasm of MKs (121). IFITM3 has been shown to interact with clathrin and αIIb and change their plasma membrane localization into lipid rafts, thereby mediating endocytic transport process in platelets (122). After administration of IFN in mice, endocytosis of fibrinogen and platelets reactivity significantly increased in an IFITM3-dependent manner. In nonviral sepsis, platelet IFITM3 expression elevated, leading to an increase fibrinogen content in platelets and thrombosis. These data support IFITM3 as a regulator of clathrin-dependent endocytosis in platelets, hyperreactivity, and thrombosis during inflammatory stress (122).

## 3.2 Clathrin independent endocytosis

The most common non clathrin-coated pits are known as caveolae (123). Caveolae-mediated endocytosis forms flask-shaped invaginations decorated with caveolin proteins on the surface of plasma membrane (124, 125). There are three types of caveolin proteins in mammals: caveolin1, caveolin 2, caveolin 3. Caveolin 3 is muscle specific, while the other two are widely expressed in non-muscle cells (123). Human platelets contain all three types of caveolin; however, whether and how these proteins coupled to endocytosis in platelets is still unknown (126).

RhoA and Cdc42 (small G proteins from the Rho family) have been widely involved in clathrin-independent endocytic regulation and the control of cytoskeletal recombination and intracellular signaling events (123). RhoA recruits the actin machinery to produce membrane invaginations in the process of endocytosis (16). Deficiency of RhoA not only significantly inhibits platelet aggregation, granule secretion, spreading on fibrinogen and other coagulation functions, but also induces macrothrombocytopenia (127). Meanwhile, Cdc42 plays crucial roles in platelet aggregation in response to collagen and integrin α2β1 activity (128). However, whether they showed priority for platelet endocytosis contents remains unclear and required further investigation.

### 3.2.1 The Arf GTPase family

The Ras-like, small guanosine triphosphate (GTP)–binding proteins named adenosine 5′-diphosphate–ribosylation factors (Arf's), play essential roles in intracellular trafficking (47). Based on sequence homology, they are separated into three types largely as Class I (Arfs 1–3), Class II (Arfs 4–5) and Class III (Arf6) (6). Among them, Arf6 has been most concerned in platelets. In rest platelets, Arf6 exists in an active, GTP-bound state. Upon stimulation by collagen and convulxin, the level of Arf6 in platelets rapidly decreased, by converting it to an inactive Arf6-GDP form (129). This process is regulated by the primary

signaling activated by PAR receptor and GPVI, or contact-dependent signaling by integrin αIIbβ3 (130). When switch between active and inactive state, Arf6 facilitates internalization of ligand and recycling of receptors (131). In human platelets, Arf6 regulates the internalization and function of $P2Y_1$ and $P2Y_{12}$ purinoceptors. Moreover, Arf6 can regulate Nm23-H1 activation, a nucleoside diphosphate kinase, then facilitate fission of coated vesicles in a dynamin-dependent manner during endocytosis (132). Huang et al. generated mice with Arf6 deficiency in platelets, they found that hemostasis phenotypes (e.g., aggregation, ADP secretion, tail bleeding times, and occlusion time of arterial thrombosis) were normal, yet, Arf6 deficient platelets displayed enhanced spreading on fibrinogen and accelerated clot retraction. It is worth noting that fibrinogen uptake and storage were defective in $Arf6^{-/-}$ platelets (47). Thus, Arf6 may selectively regulate endocytic trafficking of platelet αIIbβ3 and the efficacy of platelet function. Arf1 and Arf3 are more abundant than Arf6 in platelets (6). In addition, several Arf regulators such as the GAPs, GEFs, ASAP1/2, ArfGAP1/2, and ARAP1 are present in platelets (107). Their regulatory roles in platelet endocytosis required further investigations.

### 3.2.2 Toll like receptor-4

Platelets can remove pathogens quickly through toll-like receptors (TLRs), and their endocytic and phagocytic abilities proved their association with the innate immune mechanisms (133). A variety of TLRs are expressed in platelets, some of which are expressed on platelet membranes and some of which are expressed inside platelets. Previous study revealed that platelets internalized circulating platelet microparticles (PMP) through TLR-4 and TLR-4 related components (133). It has been confirmed that PMP internalization in platelets could proceeds thrombus formation. Thus blockade of TLR-4 mediated endocytosis might be a potential target for antithrombotic therapies in pathological situations.

## 3.3 Phagocytosis and pinocytosis

In certain cells, extracellular substances waiting to be internalized are gradually surrounded by the invaginations forming on the plasma membrane, and then taken up into cells. This process is called phagocytosis (123). Phagocytosis usually refers to engulfment of large particles such as dead cells or invading microbes (16). Bacteria and viruses seem to be ingested by platelets in a process akin to true phagocytosis (67, 68, 134). Platelets express some pathogen recognition receptors, such as FcγRIIa, αIIbβ3, GPIbα, CLEC-2, and DC-SIGN, which can sense bacterial and viral particles and mediate phagocytosis of platelets (65). FcγRIIa is indispensable for IgG-containing complex clearance. Platelets can phagocytose immune complexes from circulation in a FcγRIIa-dependent manner (135, 136). It has been proved that during viral infection, influenza viruses are recognized by IgG-specific antibodies and form an immune complex, which in turn is recognized by FcγRIIa, resulting in the internalization of this complex, and activating platelets (68). Besides FcγRIIa, DC-SIGN, mainly expressed in macrophages and dendritic cells, also gets involved in in phagocytosis of platelets (66). As mentioned before, DENV directly bind to platelets utilizing DC-SIGN and heparan sulfate proteoglycan as primary receptors, and mediating entry of DENV into platelets (78, 137). Moreover, previous findings support that DC-SIGN can modulate HIV phagocytosis in platelets with CLEC-2 acting as its partner (138). In conclusion, these lectins are required for efficient binding of HIV and platelets, and might play important roles in phagocytosis of virus into platelets (Figure 1).

Different from phagocytosis, pinocytosis is another form of endocytosis that small particles are taken into the cell suspended within small vesicles. Pinocytosis usually participates in the absorption of extracellular fluids. It has been reported that fibrinogen might be taken up into platelets by pinocytosis, but its specific regulatory mechanisms and influencing factors need further research (31).

# 4 Endocytic trafficking routes in platelets

Platelets take up different cargoes, but what happens next? After particles enter platelets, they will go through different transport pathways, and exerting their respective regulatory functions.

Platelets contain distinct membrane-bound compartments (endosomes) that exercise the function of "transfer stations" for internalized cargo molecules (47). Platelets have a weak ability to synthesize proteins, so most cargo is taken from plasma. OCS is a complex a system of tunneling invaginations of the plasma membrane found in platelets that connects with membrane channels and cell surfaces to control the endocytosis and exocytosis (139, 140). The role of the OCS in the regulation of platelet functions can be outlined as follows: 1. The OCS is instrumental in the endocytosis of plasma proteins and other circulating molecules for example fibrinogen and TF (23, 24). This process allows platelets to acquire components that they do not synthesize themselves. 2. The OCS aids in the internalization of bacteria such as *P. gingivalis* and viral pathogens such as HIV (67, 92). This interaction is crucial for the immune functions of platelets, contributing to their role in inflammation and defense against infections (141). 3. The potential of the OCS in clinical treatments, particularly as a drug delivery system, is highlighted by its role in the encapsulation of drugs like DOX in platelets, as demonstrated in previous studies (96). This suggests that manipulating the OCS structure could be a novel approach in drug delivery applications. It has been observed that a distinct closed canalicular system of dispersed membrane-bound structures also exist in platelets (142). During early platelets activation, the closed canalicular system fuses with the plasma membrane and becomes open, thus these closed canalicular systems might represent endosomes in platelets (16, 142).

Platelets can sort endocytosed cargoes into different endosomal compartments (Figure 2). To analyze the sorting routes in platelets, researchers use anti-Rab4 (represent early endosomes), anti-Rab11 (recycling endosomes), and anti-Rab7 (late endosomes) antibodies

as markers of different compartments. Both Rab4 and Rab11 compartments were present in platelets, but not overlapped, indicating that resting platelets contained both early and recycling endosomes (7, 8, 129). The endocytosis trafficking in platelet is time-dependent. As shown in Figure 2, internalized cargo transports through early endosomes, where it can be selected to recycling endosomes, then return back to the plasma membrane (e.g., αIIbβ3, $P2Y_{12}$, TPOR), or to multivesicular bodies (MVB) and finally enter α-granules for storage (e.g., fibrinogen, vWF, thrombospondin-1). Either directly from early endosomes or through MVB, cargoes can also transit into late endosomes, and ultimately move into dense granules or lysosomes for storage or degradation (143, 144).

In the process of platelet endocytosis transport, a series of proteins are required to facilitate membrane fusion. Soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) can mediate inter-compartmental transport in most cells, but its function in platelets is still under investigation. Cellubrevin/Vesicle-Associated Membrane Protein-3 (VAMP-3) is a v-SNARE protein localized to punctate structures within platelets. It has been reported that VAMP-3 not only regulate membrane, but also facilitate platelet aggregation and secretion (145, 146). Banerjee and colleagues have described the role of VAMP-3 in mediating endocytosis and endosomal trafficking in platelets, as well as controlling intracellular uptake/accumulation of fibrinogen and transferrin, platelet spreading, clot retraction, and proper regulation of TPOR signaling (7).

As mentioned before, TLR4 is involved in platelet endocytosis of extracellular pathogens. After the internalization of pathogens such as bacteria and viruses, platelets may also interact with other TLRs of endosomes. For example, TLR7 in the endosome recognizes single-stranded RNA and can be activated by retroviruses (147–149). Platelet TLR9 is detected both on the platelet membrane and in the endosome (150). HIV-1 pseudovirions incubation could induce endocytosis and trafficking of virions to an acidic, degradative compartment in platelets. Subsequently, platelets were activated with granule release, and platelet-leukocyte aggregate (PLA) formation through the pathway involved in activation of TLR7/TLR9 and myeloid differentiation primary response protein 88 (MyD88), as well as the participation of downstream interleukin 1 receptor associated kinase 4 (IRAK4), Akt, and IκB kinase (IKK) cascade signaling (65).

## 5 Perspectives and conclusion

In contrast with karyocytes, platelets are anucleated but relatively complex cells, with extensive endomembrane systems, and more hectic intracellular transport activities than ever thought. Endocytosis process has great impact on the up take, storage, and activity of proteins in platelet granules. This may have significant implications in clinical treatment of human diseases, as alterations in platelet granule proteins may contribute to thrombosis or bleeding. Endocytosis is a multi-step process that utilizes several routes of cargo entry/transit/exit (16), it also involved in various functions of platelets. Endocytosis might permit platelets to take up several prothrombotic and proinflammatory mediators (e.g., fibrinogen, vWF, IgG) from plasma; these messengers can then be stored into α-granules and later released during activation. Platelets endocytosis mediates surface receptors recycling: for example, integrin αIIbβ3 trafficking may affect platelet spreading and clot retraction, and internalization of both $P2Y_1$ and $P2Y_{12}$ receptors is required for resensitization of platelet responses (50). In addition, TPO receptor Mpl endocytosed by MK/platelets could regulate megakaryopoiesis, thrombopoiesis, and bone marrow homeostasis (63). Endocytosis may also enable platelets to function as immune cells since it can phagocyte bacteria and virus. Moreover, endocytic contents from the extracellular environment may provide platelets with the ability to differentially respond to corresponding physiological and pathological stimuli, thus modulating platelet interactions with other cells (16).

From the perspective of clinical disease treatment, platelet endocytosis may also play important roles. For example, in patients with nonviral sepsis, the expression of IFITM3 and contents of fibrinogen significantly increase in platelets, indicating that endocytosis mediated by IFITM3 may affect platelets reactivity and thrombosis during inflammatory stress (122). A recent report has suggested that endosomes play a crucial role in platelet activation, particularly in disease-specific contexts such as immune-mediated inflammatory diseases (151). Hydroxychloroquine (HCQ), the mainstay treatment for systemic lupus erythematosus, has been reported to function through limiting acidification of endosome in platelets (152). This underscores the considerable potential of platelet endosome research in revolutionizing diagnostics and therapeutic strategies across a spectrum of clinical disciplines. Additionally, a deeper understanding of platelet endocytosis and its integral role in platelet biogenesis is essential, not only for the validation of ex vivo platelet production for transfusion purposes but also for pioneering applications in the realms of inflammation, infection, and oncology. Circulating platelets, as temporally-regulated reservoirs, are replete with vital constituents sourced from the bone marrow and finely tuned by the circulatory environment. Looking ahead, we envision platelets, with their unique endocytic, transport, and release capabilities, as formidable candidates for the development of advanced, targeted drug delivery systems, especially in the context of clinical oncology.

In this review, we have encapsulated the latest research on platelet endocytosis, delving into the intricacies of endosome contents, the key regulatory elements, and the complex pathways of delivery. Research in the field of platelet endocytosis is still nascent, with numerous aspects of this process yet to be unraveled. As technological advancements continue to emerge, we anticipate a deeper and more comprehensive exploration of this domain. We hope that this review will provide researchers with a comprehensive insight

into the intricacies of platelet endocytosis and transport, paving the way for further discoveries.

## Author contributions

YZ: Formal Analysis, Funding acquisition, Project administration, Writing – original draft, Writing – review & editing. JD: Conceptualization, Writing – review & editing. MW: Investigation, Writing – review & editing. YL: Conceptualization, Formal Analysis, Writing – original draft.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



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# Pilot study examining anti-factor Xa levels for heparin monitoring and outcomes in patients with cerebral venous thrombosis

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**Objective:** There are no studies to date that examine the association between anti-factor-Xa (AFXa)-based heparin monitoring and clinical outcomes in the setting of cerebral venous thrombosis (CVT).

**Methods:** This pilot study included adults aged ≥18 admitted with CVT between 1 January 2018 and 1 January 2021, who were treated with unfractionated heparin (UFH) and were monitored via AFXa-based nomogram within 24 h of arrival. Comparisons were made between patients with AFXa levels within the target therapeutic range (0.25–0.5 IU/mL) and patients whose levels were not within the therapeutic range within 24 h of arrival; the time (hours) from arrival to reach the therapeutic range was also examined. Outcomes were length of stay (LOS) in the hospital, major (actionable) bleeding events, and discharge home (vs. higher acuity location). Continuous data are reported in the form of the median (interquartile range).

**Results:** Among 45 patients, treatment with UFH was initiated 2 (1–11) h after arrival, and the majority (84%) of UFH infusions did not need dose adjustment. AFXa assays were conducted every 6 (5.5–7) h. Thirty patients (67%) fell within the therapeutic range. Outcomes were similar for patients with levels within the therapeutic range vs. not: major bleeding events, 10% vs. 0% ($p = 0.54$); discharge home, 77% vs. 80% ($p = 1.0$); LOS, 5 days in each group ($p = 0.95$). There was also no association between outcomes and time to reach the therapeutic range.

**Conclusion:** Our findings demonstrate the practicability of monitoring UFH based on AFXa values in this population of patients with CVT, but reaching target AFXa levels within 24 h of arrival may not necessarily be prognostic.

KEYWORDS

**cerebral venous thrombosis, unfractionated heparin, anti-factor-Xa, monitoring, outcomes**

## 1 Introduction

Cerebral venous thrombosis (CVT) is a rare cause of stroke that occurs in less than 1 per 100,000 people annually, and although it is generally associated with favorable outcomes, it still causes significant long-term disability and approximately 10% mortality (1, 2).

CVT is treated with systemic anticoagulant therapy regardless of the presence of pre-treatment intracerebral hemorrhage (ICH) (3). American Heart Association/ American Stroke Association guidelines recommend intravenous unfractionated heparin (UFH) or subcutaneous low-molecular-weight heparin (LMWH) (4). Heparin exerts its anticoagulant effects primarily via inactivation of thrombin and factors Xa, IXa, and XIa and XIIa, preventing fibrin formation and inhibiting thrombin-induced activation of platelets and coagulation factors (5).

LMWH demonstrates predictable pharmacodynamics and pharmacokinetics, rendering routine therapeutic drug monitoring (TDM) for anticoagulation effects unindicated in the majority of patients. However, TDM may be indicated in certain patient populations receiving LMWH to enhance the predictability of optimal dosing, including patients with hepatic or renal insufficiency, pregnant patients, octogenarians, and patients with extreme body weight (<40 kg or > 150 kg) (5, 6).

An advantage of UFH over LMWH in the context of CVT is a more immediate anticoagulant effect as well as a shorter elimination half-life (5). However, reaching an optimal therapeutic dose of UFH can be challenging due to unpredictable pharmacokinetics, interpatient variability, and anarrow therapeutic index, and TDM is indicated (7).

UFH monitoring may lead to fewer dose adjustments and less time to reach the therapeutic range, whereas suboptimal or overdosing may lead to poorer outcomes, including thromboembolic complications, major hemorrhage, and thromboembolic recurrence (8–10). While direct monitoring of serum concentration of UFH is not possible, there are several surrogate tests available for UFH monitoring, including activated partial thromboplastin time (aPTT), activated clotting time, plasma heparin concentration, and anti-factor Xa (AFXa). AFXa assays have been shown to be superior to aPTT-based protocols (10–12) and are less sensitive to reagents and less influenced by external factors that are also known etiologies of CVT, such as pregnancy and the presence of lupus anticoagulant (13, 14).

To our knowledge, there is only one published study examining AFXa monitoring in the setting of CVT, but this study was performed in children (14). There are no studies to date that have primarily focused on identifying and describing the practicability of UFH monitoring specifically in the setting of adult CVT. This pilot study describes adults with CVT treated with UFH and monitored via AFXa assay and reports on the association between AFXa monitoring and clinical outcomes.

# 2 Methods

## 2.1 Design, setting, and population

This retrospective pilot study included adults (≥18 years old) who were admitted to a comprehensive stroke center between 1 January 2018 and 1 January 2021 for CVT. AFXa assays were conducted at our institution as a surrogate marker to measure the extent of anticoagulation with UFH. Patients were excluded if they did not initially receive treatment with UFH or if they did not undergo at least one documented AFXa assay within 24 h of arrival (Figure 1). The study received IRB approval with waiver of consent. The initial study population was identified through the hospital's neurology patient registry database. Data were extracted from electronic health records by clinicians (Y.P., X.T., and L.D).

## 2.2 Anticoagulation protocol

The institutional protocol for anticoagulation treatment of CVT is to begin either LMWH or UFH within 2 h of arrival; for the latter, low-intensity heparin infusion is used for stroke. The standard heparin concentration is 100 units/mL with an initial infusion rate of 12 units/kg/h, with weight-based calculations for specific infusion rate and for the heparin bolus dose (if ordered).

Coagulation monitoring for UFH via AFXa assay began at the end of 2017 and was in place throughout the study period. The anti-factor Xa heparin assay is a chromogenic assay that measures the factor Xa-neutralizing capacity of heparin (15). When antithrombin binds to factor Xa in a sample containing heparin, it forms a complex, which can lead to underestimation of the concentration of active factor Xa in the assay. In the anti-Xa assay, the goal is to measure the residual, unbound factor Xa activity in a patient's blood plasma or serum. The assay uses a substrate that is cleaved by factor Xa, leading to a color change that can be quantified. The degree of color change is directly proportional to the amount of unbound factor Xa in the sample (5).

Our hospital uses the Siemens INNOVANCE anti-Xa assay, which is an automated chromogenic anti-Xa assay for quantitatively determining the activity of UFH (16). Quality control checks are performed at the beginning of every shift to ensure the test is running consistently. AFXa assays are ordered every 6 h after initiation of UFH infusion or any dosage change; aPTT assays may have also been performed, but not at standardized intervals or to assess coagulation. Adjustments to the infusion as determined in our hospital-based guidelines are shown in Table 1. The therapeutic range for UFH is 0.25–0.5 IU/mL, with the intent to achieve a therapeutic range within 18–24 h.

## 2.3 Exposure and outcomes

AFXa values were examined in two ways: (1) whether the patient reached the therapeutic range within 24 h of arrival (yes vs. no), defined as an AFXa value between 0.25 and 0.5 IU/mL; and (2) the time (hours) from arrival to the first AFXa within the therapeutic range. The cutoff point of 24 h was based on the institutional protocol and previous studies (6).

Study outcomes were examined during the hospitalization period and included major and minor bleeding events, other complications (e.g., ischemic or hemorrhagic stroke, intracerebral hemorrhage secondary to acute stroke treatment, groin puncture, or hospital complications/events), LOS in the hospital (days), discharge disposition, and in-hospital mortality. Bleeding events were defined *a priori* and extracted from records for this study by research clinicians (Y.P., X.T., and L.D). Major bleeding events were considered if there was discontinuation of the anticoagulant, a reversal agent was used, or the patient needed transfusion or surgery. Minor bleeding was defined as overt bleeding that was not actionable (the patient experienced symptoms but no action was needed, i.e., no discontinuation of anticoagulant, no use of a reversal agent, and no blood transfusion or surgery required). Discharge disposition was categorized as home/

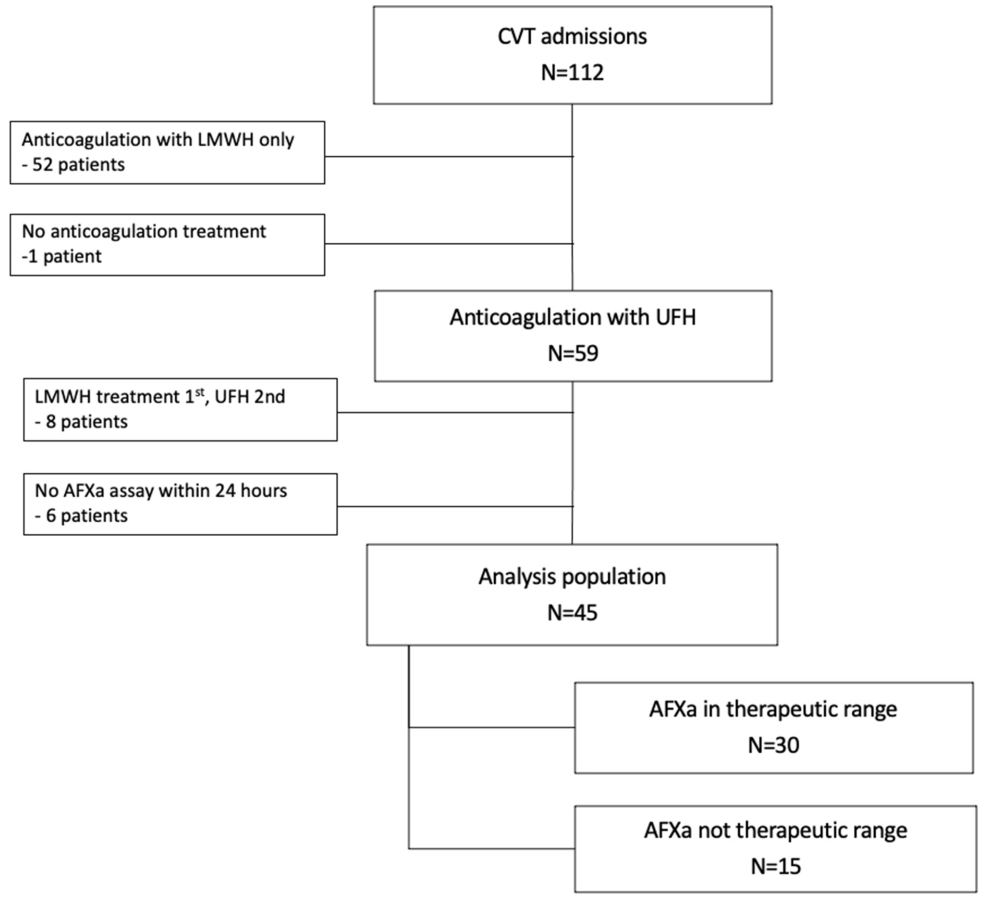


FIGURE 1
Study population. CVT, cerebral venous thrombosis; UFH, unfractionated heparin; LMWH, low molecular weight heparin; AFXa, anti-factor-Xa.

TABLE 1 Hospital-based protocol for heparin rate adjustment based on anti-factor Xa (AFXa) value.

| AFXa value (IU/mL) | Stop infusion | Bolus | Rate change (units/kg/h) | Repeat assay in: |
|---|---|---|---|---|
| Less than 0.15 | No | No | ↑ 3 units/kg/h | 6 h |
| 0.15–0.24 | No | No | ↑ 2 units/kg/h | 6 h |
| 0.25–0.5 | No change (Therapeutic Range) | | | 6 h* |
| 0.51–0.65 | No | No | ↓ 1 unit/kg/h | 6 h |
| 0.66–0.8 | 60 min | No | ↓ 2 units/kg/h | 6 h |
| 0.81 or higher | 120 min | No | ↓ 3 units/kg/h | 6 h |

*Repeat every 24 h if two consecutive assays are in the therapeutic range. kg/h, kilograms per hour.

home health care; care facility (including rehabilitation and long-term care facilities); or morgue.

## 2.4 Statistical analysis

All analyses were performed using SAS (Cary, NC) version 9.4. The threshold for statistical significance was $\alpha < 0.20$; this level of significance is appropriate for small sample sizes and pilot studies rather than the conventional $\alpha < 0.05$ (17, 18). Continuous data are presented in the form of the median (interquartile range). There was no imputation of missing data. The associations between outcomes and achievement of AFXa within the therapeutic range within 24 h (yes vs. no) were analyzed via chi-square tests (categorical outcomes) and a Wilcoxon rank-sum test (LOS in the hospital). The associations between outcomes and time (hours) to reach the therapeutic range were analyzed via Wilcoxon rank sum tests (categorical outcomes) and the Spearman correlation coefficient (LOS).

# 3 Results

## 3.1 Patients

There were 45 patients with CVT in the analysis population (Figure 1). The median age was 42 (34–57) years, and the majority of patients were female (53%), transferred in (69%), presented with headache (76%), and had multiple veins/sinuses involved (Table 2). Outcomes were generally favorable. The majority of patients (78%) were discharged home, 20% were discharged to a care facility, and one patient died. There were three documented major bleeding events (6%); surgery, a reversal agent, and discontinuation of the UFH

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TABLE 2 Patient characteristics.

| Covariate | AFXa in the therapeutic range | | | |
|---|---|---|---|---|
| | Overall ($n = 45$) | Yes 30 (67%) | No 15 (33%) | $p$-value |
| Age, median (IQR) | 42 (34–57) | 40.5 (34–54) | 46 (39–65) | 0.36 |
| Male sex | 21 (46.7) | 15 (50.0) | 6 (40.0) | 0.53 |
| White race | 25 (55.6) | 16 (53.3) | 9 (60.0) | 0.67 |
| Sinus involvement* | | | | |
| Superior sagittal sinus | 20 (44.4) | 15 (50.0) | 5 (33.3) | 0.29 |
| Straight sinus | 4 (8.9) | 2 (6.7) | 2 (13.3) | 0.59 |
| Transverse sinus | 30 (66.7) | 19 (63.3) | 11 (73.3) | 0.50 |
| Sigmoid sinus | 29 (64.4) | 18 (60.0) | 11 (73.3) | 0.38 |
| Jugular vein | 14 (31.1) | 8 (26.7) | 6 (40.0) | 0.50 |
| Other | 11 (24.4) | 8 (26.7) | 3 (20.0) | 0.73 |
| Endovascular therapy | 22 (48.9) | 17 (56.7) | 5 (33.3) | **0.14** |
| Signs/symptoms* | | | | |
| Headache | 34 (75.6) | 22 (73.3) | 12 (80.0) | 0.73 |
| Hemorrhagic CVT | 6 (13.3) | 5 (16.7) | 1 (6.7) | 0.65 |
| Seizure | 14 (31.1) | 11 (36.7) | 3 (20.0) | 0.32 |
| Altered mentation | 8 (17.8) | 6 (20.0) | 2 (13.3) | 0.70 |
| Nausea or vomiting | 4 (8.9) | 3 (10.0) | 1 (6.7) | 1.0 |
| Motor impairment | 8 (17.8) | 5 (16.7) | 3 (20.0) | 1.0 |
| Sensory or visual | 9 (20.0) | 6 (20.0) | 3 (20.0) | 1.0 |
| Transferred in | 31 (68.9) | 21 (70.0) | 10 (66.7) | 1.0 |

IQR, interquartile range; CVT, cerebral venous thrombosis. *More than 1 allowed, will not total 100%. Bolding denotes statistical significance at $p < 0.20$.

infusion were each required in one case. Four additional patients had a minor bleeding event. One patient developed an additional complication, a groin hematoma, following intra-arterial therapy. The median LOS in the hospital was 5 (4–8) days.

The median time to initiate UFH was 2 (1–11) h after arrival. Approximately 24% of patients received an initial bolus, with a median of 2,000 (1,000–3,000) units. The median continuous infusion was 250 (250–250) units/kg. The majority of patients (84%) did not need UFH volume adjustment, and the majority of heparin infusions (84%) did not need volume adjustment. Most patients (84%) were transitioned to LMWH, approximately 27 (17–61) h after arrival. Fifteen patients received LMWH within 24 h of arrival.

AFXa assays were conducted every 6 (5.5–7) h; there were 4 (2–8) draws per patient. The median time from arrival to the first AFXa assay was 7.5 (3–9) h.

## 3.2 Therapeutic range within 24 hours

There were 30 patients (66.7%) who were within the therapeutic range within 24 h of arrival. The median time to reach the therapeutic range was 9 (5–15.5) h after initiation of UFH, or 14 (9–20) h after arrival. The heparin infusion volume was adjusted for three patients (10%) who were within the therapeutic range within 24 h of arrival and four patients (27%) who were not within the therapeutic range within 24 h of arrival.

Patient demographics and presenting characteristics were similar for patients who were in the therapeutic range vs. those who were not, with the exception that patients in the therapeutic range were more likely to receive endovascular therapy than those who were not (56.7% vs. 33.3%, $p = 0.14$) (Table 2).

More than half (8 of 15) of the patients who were not within the therapeutic range within 24 h did not reach the therapeutic range at any point during their hospital stay. Among the 15 patients who were not within the therapeutic range within 24 h, 8 patients were supratherapeutic (AFXA >0.81), 3 patients were subtherapeutic (AFXA <0.15), and 4 patients had AFXa values that were outside the therapeutic range but were in the range 0.15–0.49 or 0.51–0.80.

## 3.3 Association with outcomes

There was no association between study outcomes and whether the patient achieved AFXa values within the therapeutic range within 24 h or not (Table 3). Major bleeding events were reported in 10% ($n = 3$) of patients who reached the therapeutic range vs. 0% for those who did not within within 24 h of arrival ($p = 0.54$); the rate of home discharge was 77% for those who were within the therapeutic range vs. 80% who were not ($p = 1.0$); and LOS in the hospital was 5 days for each group ($p = 0.95$). Similarly, for the subset of 22 patients who underwent endovascular therapy, there was no association between study outcomes and whether the patient achieved AFXa values within the therapeutic range within 24 h or not (Table 3).

There were also no major differences in outcomes for patients based on the degree to which patients' AFXa values fell within the therapeutic range within 24 h (Table 4).

There was also no association between study outcomes and the amount of time taken (hours) to reach the therapeutic range. The median time to reach the therapeutic range was 11 (8–15) h for those discharged home vs. 14 (8–20) h for discharge to a care facility or morgue ($p = 0.49$), and 13 (9–20) h for patients who developed a major bleeding event vs. 12 (8–16) h for those without a major bleeding event ($p = 0.68$). There was also no correlation with LOS in the hospital ($p = 0.77$).

# 4 Discussion

In this pilot study of patients with CVT, we examined whether dose-adjusted monitoring of UFH using an AFXa assay was associated with clinical outcomes (LOS, major bleeding events, or favorable discharge disposition). Outcomes were similar for patients whose levels were within the therapeutic range and those who were not within the therapeutic range within 24 h of arrival. Taking less time to reach therapeutic AFXa values was also not associated with better outcomes, although poor outcomes were uncommon. One interpretation of our findings is that the results of AFXa monitoring within 24 h may not be prognostic for patients with CVT, as there was no association with outcomes and most patients were within the therapeutic range within 24 h without requiring dose adjustment.

To our knowledge, there is only one other published study examining AFXa monitoring in the setting of CVT. Saini et al.

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TABLE 3 Association between therapeutic anti-factor Xa (AFXa, 0.25–0.5 IU/mL) within 24 h of arrival and outcomes.

| Outcome | Therapeutic AFXa: all patients ($n$ = 45) | | | Therapeutic AFXa: endovascular therapy subset ($n$ = 22) | | |
|---|---|---|---|---|---|---|
| | Yes 30 (67%) | No 15 (33%) | $p$-value | Yes 17 (77%) | No 5 (23%) | $p$-value |
| Discharge disposition, $n$ (%) | | | 0.77 | | | 0.76 |
| Home | 23 (76.7) | 12 (80.0) | | 11 (64.7) | 4 (80.0) | |
| LTAC/Rehab | 6 (20.0) | 3 (20.0) | | 5 (29.4) | 1 (20.0) | |
| Died | 1 (3.3) | 0 (0) | | 1 (5.9) | 0 (0) | |
| Major bleed, $n$ (%) | 3 (10.0) | 0 (0) | 0.54 | 2 (11.8) | 0 (0) | 1.0 |
| Minor bleed, $n$ (%) | 2 (6.7) | 2 (13.3) | 0.59 | 1 (5.9) | 1 (20.0) | 0.41 |
| Median LOS (IQR) | 5 (3–9) | 5 (4–8) | 0.95 | 6 (4–12) | 5 (4–9) | 0.69 |

*Among 15 patients not in the therapeutic range, 6 patients were supratherapeutic (AFXA > 0.50), 4 patients were subtherapeutic (AFXA < 0.25), and 5 patients had AFXa values that were both super- and subtherapeutic. LTAC, long-term acute care; IQR, interquartile range. Major bleeds were actionable, whereas minor bleeds required no action.

TABLE 4 Association between anti-factor Xa values (IU/mL) within 24 h of arrival and outcomes.

| Outcome | Therapeutic | Not in the therapeutic range | | | $p$ value |
|---|---|---|---|---|---|
| | AFXa 0.25–0.50 $N$ = 30 | AFXa <0.15 $N$ = 3 | AFXa >0.81 $N$ = 8 | AFXa >0.15 and < 0.81 $N$ = 4 | |
| Discharge disposition, $n$ (%) | | | | | |
| Home | 23 (76.7) | 2 (66.7) | 7 (87.5) | 3 (75.0) | 0.98 |
| LTAC/Rehab | 6 (20.0) | 1 (33.3) | 1 (12.5) | 1 (25.0) | |
| Died | 1 (3.3) | 0 (0.0) | 0 (0) | 0 (0) | 0.92 |
| Major bleed, $n$ (%) | 3 (10.0) | 0 (0.0) | 0 (0) | 0 (0) | 0.66 |
| Minor bleed, $n$ (%) | 2 (6.7) | 1 (33.3) | 1 (12.5) | 0 (0) | 0.41 |
| Median LOS (IQR) | 5 (3–9) | 8 (6–16) | 5 (3.5–7) | 4.5 (3–6) | 0.36 |

LTAC, long-term acute care; IQR, interquartile range. Major bleeds were actionable, whereas minor bleeds required no action.

retrospectively compared aPTT and AFXa assays in 95 children with CVT and reported that peak AFXa and peak aPTT values were not predictive of clinical outcomes, including major bleeding events, which were reported in 5.3% of cases (14). The major bleeding event rate in our adult population (5.9%) was similar.

UFH comprises a diverse blend of glycosaminoglycans that interact with antithrombin (AT) through a pentasaccharide, promoting the deactivation of thrombin and various clotting components. In this context, AT is not directly competing with factor Xa in the assay. Instead, the role of AT is to enhance the inhibitory effect of heparin on factor Xa, resulting in lower levels of unbound factor Xa, which can then be measured by the assay (5). AT enhances the action of heparin in inhibiting factor Xa in the anti-Xa assay by forming a ternary complex with heparin and factor Xa. This assay is used to indirectly measure the residual factor Xa activity in the presence of heparin, providing valuable information for monitoring and adjustment of anticoagulant therapy.

Renal impairment is an important confounding factor because the blood concentration and clearance rate of UFH depend on renal function (7). No patients in our study had renal failure. One patient with a mild AKI (GFR of 57) had a supratherapeutic AFXa while on UFH, and the infusion was stopped as indicated in the guidelines; this patient was transitioned to an alternative anticoagulant. For the purposes of this study, we focused on AFXa levels in patients receiving UFH. Although the issue falls outside the scope of this study, our current clinical practice is to avoid using LMWH in patients with a GFR of less than 30.

In the setting of traumatic injury, findings are inconsistent on whether AFXa monitoring is associated with outcomes. When compared to historical controls, a benefit from AFXa-guided dosing of LMWH has been observed (19, 20). Singer et al. studied 131 trauma patients admitted to the ICU and reported that 35% initially achieved prophylactic levels based on AFXa assay, 25% required a dose adjustment, and 39% did not reach therapeutic levels, but there was a reduction in VTE rates with AFXa-guided dosing to 7.1%, down from 20.5% in historical controls (20). Ko et al. reported lower rates of symptomatic VTE with AFXa dose adjustment of LMWH from 7.6% in historical controls to 1.1% in a study of 205 trauma patients. In contrast, Karcutskie et al. retrospectively studied 792 trauma patients and found no difference in outcomes, including VTE rates with AFXa-guided dosing of thromboprophylaxis with LMWH vs. fixed dosing (6.8% vs. 6.0%). Moreover, 48% of patients never reached prophylactic levels even with TDM (21). In our study, 33% of patients were not within the target therapeutic range within 24 h, and 18% of patients never reached target AFXa levels. While studies in trauma patients are not directly applicable to our population, they shed some light on

whether outcomes are improved by achieving higher rates of therapeutic or prophylactic anticoagulation based on AFXa dose adjustments.

Our study does have important limitations to consider. Primarily, these results should be interpreted within the scope of a pilot study because of the small sample size and the fact that all patients were treated at a single center. We were unable to adjust for covariates, and residual confounding was not assessed. A larger, better-powered study would certainly need to employ a multicenter, multiyear design. Second, we did not collect data on resolution of symptoms or modified Rankin scale at discharge. However, discharge disposition was examined, which is an important indicator of clinical outcomes. Third, there was a high transfer rate of 69%, and we acknowledge the related limitations, including not being able to evaluate heparin treatment prior to arrival at our institution. There were seven patients who were therapeutic before starting UFH, six of whom were transfers. Moreover, UFH adjustments may vary by institution, and given the high rate of transfer, variability may have been introduced prior to definitive treatment at our comprehensive stroke center. Fourth, timing (hours) to reach the therapeutic range was calculated as the time from arrival to the first AFXa assay falling within the therapeutic range, rather than from the time when the first dose of UFH was administered, partly because it was not known whether heparin treatment was received at the transferring facility. Fifth, 15 patients were transitioned from UFH to LMWH within 24 h of arrival. A comparison between patients who received UFH, LMWH, or both anticoagulants is provided in Supplementary Table S1. There were no clinical or demographic differences among patients based on the choice of anticoagulant, and there was no difference in outcomes for patients receiving LMWH, UFH, or both. Finally, patients were presumed to have undergone UFH dose adjustments based on AFXa values.

## 5 Conclusion

In this novel pilot study of adults with CVT, there was no association between clinical outcomes and reaching therapeutic levels on UFH, as determined by monitoring of AFXa levels. There was also no association between the time to reach therapeutic AFXa levels and clinical outcomes. Most CVT patients achieved target therapeutic AFXa levels within 24 h of their arrival, and most patients had favorable outcomes. Our findings demonstrate the practicability of monitoring UFH based on AFXa assay in this population of patients with CVT, but reaching target AFXa levels within 24 h of arrival may not necessarily be prognostic.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

## Ethics statement

The studies involving humans were approved by HealthONE institutional review board #779117. The studies were conducted in accordance with the local legislation and institutional requirements. The Ethics Committee/Institutional Review Board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because this was a minimal-risk retrospective observational study.

## Author contributions

YP: Data curation, Writing – original draft. KS: Formal analysis, Methodology, Software, Writing – original draft. CB: Writing – review & editing. XT: Data curation, Writing – review & editing. DB-O: Writing – review & editing, Project administration, Supervision. RB: Conceptualization, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2024.1317246/full#supplementary-material

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# Case Report: First attempt by off-label use of tenecteplase to treat acute extensive portal venous system thrombosis

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Acute extensive portal venous system thrombosis (PVST) can cause lethal complications. Herein, we have for the first time reported the use of anticoagulation combined with systemic thrombolysis by tenecteplase in a male patient with a diagnosis of acute extensive PVST but without liver cirrhosis. After thrombolytic therapy, abdominal pain obviously alleviated. However, urinary bleeding developed, which was reversible by stopping thrombolytic drugs. Finally, this case developed cavernous transformation of the portal vein without portal venous recanalization. In future, the efficacy and safety of tenecteplase should be explored in acute extensive PVST cases.

KEYWORDS

portal venous system thrombosis, thrombolysis, tenecteplase, bleeding, case report

## Introduction

Portal venous system thrombosis (PVST) is a common complication of liver cirrhosis and associated with the severity and progression of liver cirrhosis, but is relatively rare in patients without liver cirrhosis (1). PVST is not only located in the main portal vein, but also extends to mesenteric and splenic veins (2). Occlusive PVST can cause intestinal ischemia and portal hypertension related complications, such as gastroesophageal variceal bleeding (3). Anticoagulation is the first-line therapeutic choice in patients with acute PVST, and thrombolysis is considered when this condition is aggravated (4). Thrombolytic agents include streptokinase, urokinase, and tissue type plasminogen activator (t-PA) (5). They are often administered intravenously or locally through a catheter. Tenecteplase is a variant of native t-PA produced by recombinant DNA technology (6). It is approved by the United States Food and Drug Administration for the treatment of acute myocardial infarction (AMI) and is being attempted for the treatment of stroke. To the best of our knowledge, tenecteplase has not been given in cases with PVST yet. Herein, we reported an uncommon case of acute extensive non-cirrhotic PVST who received anticoagulation in combination with systemic thrombolysis by tenecteplase.

## Case presentation

On July 16, 2023, a 23-year-old male was admitted to our department due to abdominal pain with fatigue and no defecation for a duration of 12 days. He had a history of COVID-19 in February 2023. He had been studying in Australia for 3 years

and returned to China on July 15. His body temperature was normal. Physical examination showed upper abdominal tenderness without rebound tenderness. Laboratory tests showed that fibrinogen (FIB) was 7.95 g/L (reference range: 2.00–4.00 g/L), prothrombin time (PT) was 14.7 s (reference range: 9.0–13.0 s), international normalized ratio (INR) was 1.3 (reference range: 8–1.2), C-reactive protein (CRP) was 63.98 mg/L (reference range: <10 mg/L), and total cholesterol was 6.41 mmol/L (reference range: 0–5.17 mmol/L). Intestinal obstruction was excluded by abdominal x-ray scans, but unenhanced computed tomography (CT) scans showed high density lesion in the superior mesenteric vein (Figure 1).

On July 17, 2023, contrast-enhanced CT scans showed that the portal vein, superior mesenteric vein, and splenic vein thrombus were occlusive (Figure 2A). Gastrointestinal endoscopy showed erosive gastritis without gastroesophageal varices. Laboratory tests showed that D-dimer was 13.47 mg/L (reference range: 0.00–0.55 mg/L), fibrinogen degradation products (FDP) was 30.11 μg/ml (reference range: 0.0–5.0 μg/ml), FIB was 6.36 g/L, PT was 15.3 s, INR was 1.36, CRP was 52.34 mg/L, and procalcitonin (PCT) was 0.14 ng/ml (reference range: 0–0.05 ng/ml). Antithrombin Ⅲ and homocysteine levels were within their reference ranges. Considering a diagnosis of acute extensive PVST, he received subcutaneous injection of enoxaparin 40 mg every 12 h for anticoagulation.

On July 18, 2023, he had a fever with the highest body temperature of 39.5°C, and abdominal pain remained. Laboratory tests showed that CRP was 46.89 mg/L and PCT was 0.27 ng/ml. He received intravenous antibiotics.

On July 19, 2023, his highest body temperature was about 38.5°C. Laboratory tests showed that D-dimer was 15.35 mg/L, FDP was 36.32 μg/ml, FIB was 5.54 g/L, PT was 14.1 s, INR was 1.24, and CRP was 44.56 mg/L. Plasma protein C and S activities were within their reference ranges. He underwent abdominal vascular ultrasound showing extensive thrombosis in the portal vein, superior mesenteric vein, and splenic vein.

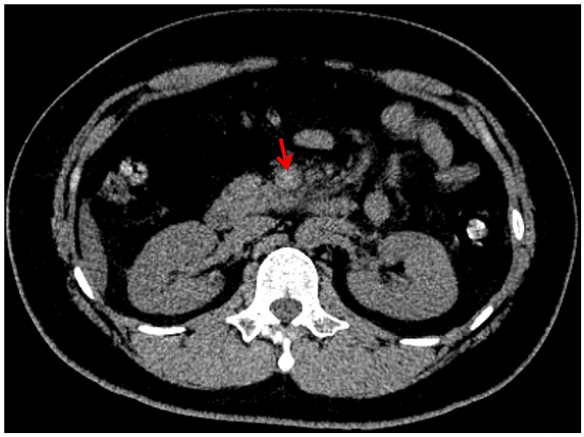

FIGURE 1
Unenhanced CT image showing high density lesion in superior mesenteric vein.

Thus, continuous intravenous infusion of urokinase 300,000 u every 12 h for systemic thrombolysis was added.

On July 20, 2023, COVID-19 antigen was positive. Laboratory tests showed that antiphospholipid syndrome antibodies were within their reference ranges. His body temperature dropped to 37.9°C, and antibiotic therapy was stopped. To increase the possibility of recanalizing PVST, continuous intravenous infusion of tenecteplase 16 mg every day was employed.

On July 21, 2023, plasminogen activator inhibitor type 1 (PAI-1) gene test showed 4G/5G heterozygous mutation.

On July 22, 2023, a painful patchy ecchymosis with a diameter of 5 cm occurred at the site of enoxaparin injection. Laboratory tests showed significant increases in D-dimer and FDP levels. D-dimer was 40 mg/L, FDP was 104.96 μg/ml, FIB was 5.54 g/L, PT was 13.3 s, and INR was 1.17. Subcutaneous injection of enoxaparin was replaced by 10 mg of rivaroxaban orally twice daily for anticoagulation.

On July 23, 2023, he had gross hematuria about 100 ml in the morning, accompanied by right lumbar discomfort, dizziness, and left upper limb numbness. He was conscious and answered fluently. Physical examination showed the absence of neck stiffness, abnormalities in muscle strength and tension of the limbs, or percussion pain in the renal and ureteral walking areas. Head CT scans did not show abnormal lesions. Routine urine tests showed a large number of red blood cells under the microscope. Anticoagulation and thrombolysis were immediately stopped, and the patient was closely observed.

On July 24, 2023, abdominal pain significantly alleviated without gross hematuria. Laboratory tests showed that D-dimer was 36.38 mg/L, FDP was 58.24 μg/ml, FIB was 4.96 g/L, PT was 13.2 s, and INR was 1.16. Abdominal vascular ultrasound was performed again, which still showed the presence of PVST. After discussing with the patient and his family, anticoagulation and thrombolysis were resumed.

On July 26, 2023, repeated contrast-enhanced CT scans showed that portal vein, superior mesenteric vein, and splenic vein were still occlusive with the development of collateral vessels (Figure 2B). Laboratory tests showed that D-dimer was 5.89 mg/L, FDP was 9.92 μg/ml, FIB was 4.96 g/L, PT was 14.7 s, and INR was 1.3. He discharged with oral rivaroxaban.

At his last visit on January 5, 2024, he did not have any other complaint. Repeated contrast-enhanced CT scans showed that portal vein could not be identified, but was replaced by multiple collateral vessels (Figure 2C).

## Discussion

Gastroenteritis, irritable bowel syndrome, pancreatitis, diverticulitis, appendicitis, and abdominal tumors are common causes of abdominal pain in the gastroenterology (7). Acute PVST is a rare disease that can also present with varying degrees of abdominal pain, which depends on the location and grade of venous occlusion. Portal hypertension develops when PVST progresses to a chronic stage (8).

PVST is often secondary to a single or multiple risk factors according to the Virchow's triad. Systemic risk factors, such as

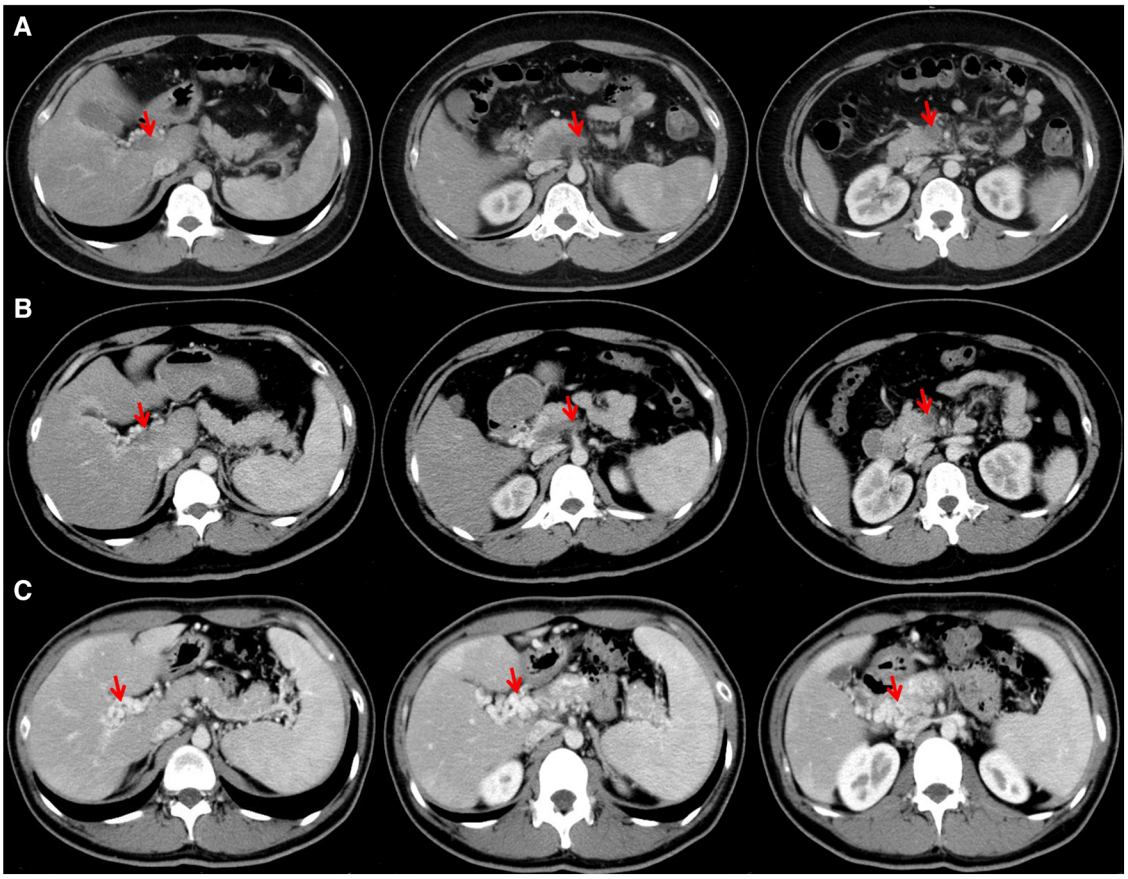


FIGURE 2
Contrast-enhanced CT images performed on July 17 (A) and July 26 (B) showing thrombosis in portal vein, splenic vein, and superior mesenteric vein, and those on January 5, 2024 (C) showing cavernous transformation of the portal vein.

myeloproliferative neoplasms, prothrombin gene G20210A mutation, antiphospholipid syndrome, and local risk factors, such as intra-abdominal surgery and inflammatory conditions, are well recognized in non-cirrhotic PVST, but were not found in our case (2, 3). Notably, a history of COVID-19 was discovered in our case. Incidence of splanchnic vein thrombosis in patients with COVID-19 is about 0.6% (9). It has been studied that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the culprit for COVID-19, could be associated with splanchnic vein thrombosis (10). SARS-CoV-2 can bind to angiotensin-converting enzyme 2 receptors on epithelial cells, thereby leading to vascular endothelial dysfunction. Additionally, immune response secondary to infection can release a large number of inflammatory factors, such as interleukin-2, interleukin-6, and tumor necrosis factor, which can cause hypercoagulability and hypo-fibrinolysis state (11, 12).

PAI-1 plays an important role in fibrinolysis system. Elevated PAI-1 generally promotes the development of venous thrombosis by inhibiting t-PA and urinary type plasminogen activator (u-PA) (13). PAI-1 gene is located on human chromosome 7, and the 4G allele binds to enhancer region, which will increase the transcription of PAI-1 gene and the PAI-1 level (14). Genetic polymorphism is theoretically associated with splanchnic vein thrombosis, but it is still disputed. PAI-1 4G/5G may increase the risk of venous thrombosis compared with PAI-1 5G/5G (15). However, gene polymorphism may not increase the risk of splanchnic vein thrombosis, despite 4G allele is associated with inherited and acquired thrombophilia (16).

Imaging tests are needed for a definite diagnosis of PVST in patients who present with abdominal pain and have potential risk factors for PVST (17). Ultrasound is the first-line imaging method, but the accuracy of diagnosing thrombosis within mesenteric veins may be affected by ascites, obesity, and bowel gas (1, 17). Contrast-enhanced cross-sectional imaging tests can further diagnose PVST who have failed by ultrasound, and can identify the presence of cirrhosis and malignant tumors (18).

Severe PVST may lead to intestinal ischemia and necrosis, and even death (19). In order to avoid PVST related complications with their high mortality, early anticoagulation therapy has been recommended and should be maintained for at least 6 months (4, 19). Thus, our patient received anticoagulation immediately when PVST was diagnosed. Thrombolysis is considered to further improve revascularization, and its efficacy and safety have been discussed in previous studies (20–22). Local thrombolysis is preferred, and streptokinase, urokinase, and alteplase are often selected (23). As previously reported by our group, urokinase was

given in patients with acute symptomatic PVST who did not respond to anticoagulation alone (24, 25). It is worth to note that we were the first to report the use of thrombolytic therapy for acute PVST by tenecteplase. We chose the dosage of tenecteplase according to the design of a Chinese study about its pharmacokinetics and tolerability, which suggested that tenecteplase at a dosage of 15–20 mg should be effective and safe (26). Compared with alteplase, tenecteplase has longer half-life and higher fibrin specificity, and is more resistant to PAI-1, which can enhance clots degradation (27). Unfortunately, our patient did not achieve venous revascularization by tenecteplase during hospitalization, which might be associated with increased fibrinolytic resistance secondary to COVID-19 (28). It could be explained by the potential mechanism that COVID-19 causes inflammatory factor storm, increasing the PAI-1 level, which is a hypo-fibrinolysis state, and then solidifying the clot structure (29). Notably, it is seemingly counterintuitive that our patient had a high level of D-dimer, which might be due to adequate tissue-level hyperfibrinolysis but insufficient systemic hyperfibrinolysis (30). Additionally, it has been shown that tenecteplase could overcome fibrinolytic resistance (29), but our patient was infected with SARS-CoV-2 during the period of acute thrombosis, which might offset the benefit of tenecteplase on fibrinolytic resistance.

Our patient had hematuria during thrombolytic period and adverse reaction disappeared by discontinuing tenecteplase. Patients treated with tenecteplase had a lower rate of non-cerebral bleeding than those treated with alteplase, and genitourinary bleeding rate in 24 h and 30 days was 0.26% and 0.38%, respectively (31, 32). However, these data were derived from AMI patients. Therefore, our case provided new data regarding use of tenecteplase for acute extensive PVST. In addition, continuously intravenous infusion of tenecteplase as a mode of administration in this patient might be a potential cause of bleeding event.

In conclusion, the probability of acute PVST should not be neglected in previously healthy patients presenting with acute abdominal pain. Considering serious complications of acute PVST, early antithrombotic treatment is needed. To the best of our knowledge, this case should be the first to use tenecteplase for the treatment of acute PVST. However, we have to acknowledge that the risk of bleeding during the continuous intravenous infusion of tenecteplase should be cautiously concerned even in the case of no definite risk factors of bleeding. In future, large-scale studies should be necessary to evaluate the optimal dosage and duration of tenecteplase in such patients and clarify how to monitor and manage its adverse effects in clinical practice.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

## Ethics statement

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

## Author contributions

QL: Writing – original draft. YP: Writing – review & editing. YZ: Writing – review & editing. XQ: Conceptualization, Writing – review & editing.

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The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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# Increased miR-6132 promotes deep vein thrombosis formation by downregulating FOXP3 expression

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**Background:** Deep vein thrombosis (DVT) is associated with aberrant gene expression that is a common peripheral vascular disease. Here, we aimed to elucidate that the epigenetic modification of forkhead box protein 3 (FOXP3) at the post-transcriptional level, which might be the key trigger leading to the down-regulation of FOXP3 expression in DVT.
**Methods:** In order to explore the relationship between microRNAs (miRNAs) and FOXP3, mRNA and microRNA microarray analysis were performed. Dual luciferase reporter assay was used to verify the upstream miRNAs of FOXP3. Quantitative real-time polymerase chain reaction, flow cytometry and Western blot were used to detect the relative expression of miR-6132 and FOXP3. Additionally, DVT models were established to investigate the role of miR-6132 by Murine Doppler Ultrasound and Hematoxylin-Eosin staining.
**Results:** Microarray and flow cytometry results showed that the FOXP3 expression was decreased while miR-6132 level was increased substantially in DVT, and there was significant negative correlation between miR-6132 and FOXP3. Moreover, we discovered that overexpressed miR-6132 reduced FOXP3 expression and aggravated DVT formation, while miR-6132 knockdown increased FOXP3 expression and alleviated DVT formation. Dual luciferase reporter assay validated the direct binding of miR-6132 to FOXP3.
**Conclusion:** Collectively, our data elucidate a new avenue through which up-regulated miR-6132 contributes to the formation and progression of DVT by inhibiting FOXP3 expression.

KEYWORDS
deep vein thrombosis, FOXP3, miR-6132, immune response, post-transcriptional regulation

## Introduction

Deep vein thrombosis (DVT) is the formation or presence of a thrombus in the deep veins, that most commonly occurs in the large veins of the legs or pelvis. Pulmonary embolism (PE) is a terrible complication of DVT and the primary contributor to mortality, PE and DVT are known as venous thromboembolism (VTE) (1, 2). The annual incidence of DVT is about 1 per 1,000 population and it seriously affects the health and quality of patients' life (3, 4). Incidence is steadily rising due to association

with many types of risk factors, such as population ageing, genetics, obesity, lifestyle, malignancy, autoimmune diseases, and infection (5, 6). Nevertheless, autoimmune diseases are frequently associated with venous thrombotic events (7). In order to find new targets for the treatment of DVT, it deeply desiderates to reveal the underlying molecular mechanism of DVT formation.

In previous studies, it was found that the proportion of Regulatory T cells (Tregs) subgroups in DVT model rats were significantly reduced (8). Tregs are a subset of T-cells that inhibit immune responses and play an important role in maintaining immune tolerance. Meanwhile, Tregs also can negatively regulate varieties of physiological and pathological immune responses (9). As well known, Tregs express surface molecules of CD4 and CD25, secret anti-inflammatory cytokines represented by IL-10 or TGF-β1 (10, 11). However, the most important transcription factor for Tregs is Forkhead box protein 3 (FOXP3), which plays an important role in their development and differentiation (12). FOXP3 is a 48 kD protein composed of 431 amino acids and is also a member of the fork head transcription factor family (13, 14). The human gene of FOXP3 consists of 11 exons and exists in the p arm of the X chromosome (Xp11.23), while the mouse gene at X A1.1 is equivalent to that of the human gene (15, 16). It has also been confirmed that FOXP3 signaling pathways regulate the process of immune, but the role of FOXP3 and its upstream regulatory factors remain ambiguous in the formation of DVT.

MicroRNAs (miRNAs) are small endogenous noncoding RNAs (typically 21–23 nucleotides in length) that negatively regulate gene expression via recognition of cognate sequences and interference of transcriptional, translational or epigenetic processes (17–19). As posttranscriptional regulators of gene expression, miRNAs have huge potential biological functions in the physiological and pathological processes of various diseases (20, 21). Interestingly, dysregulation of miRNAs expression was shown to be associated with various of cardiovascular diseases, such as atherosclerosis and DVT (22, 23). As a result, we speculated that certain miRNAs acting on FOXP3 might represent novel targets for DVT treatment.

In this study, we observed that FOXP3 expression was decreased and miR-6132 was increased in DVT patients. Notably, miR-6132 bound to its 3′UTR and thus effectively suppressed FOXP3 expression. Meanwhile, we found that increasing miR-6132 could aggravate DVT formation by inhibiting FOXP3 expression, while inhibiting miR-6132 could alleviate the formation of DVT by enhancing FOXP3 expression. Therefore, the newly discovered miR-6132/FOXP3 axis might offer new strategies for DVT treatment.

# Methods and materials

## Patients

A total of 36 patients diagnosed with symptomatic first idiopathic DVT of the lower limbs, and 36 control subjects in the Affiliated Hospital of Shandong University of Traditional Chinese Medicine from July 2018 to July 2022 were enrolled in this study. The involved DVT patients were made a definite diagnosis by Color Doppler Ultrasound and lower extremity angiography without a history of diabetes mellitus, hypertension or other chronic diseases (Table 1). This study was written informed consent was obtained from all participants and approved by the Ethics Committee of Shandong University of Traditional Chinese Medicine.

TABLE 1 Baseline characteristics of DVT patients and healthy controls.

| Characteristics | Control ($n = 36$) | DVT ($n = 36$) |
|---|---|---|
| Age, years (mean ± SD) | 54.1 ± 8.7 | 57.3 ± 9.9 |
| Gender, females/males | 17/19 | 16/20 |
| Recent immobilization/surgery | 0 | 0 |
| Anti-coagulants or platelet-inhibitors | 0 | 0 |
| Hormone | 0 | 0 |
| Smoking | 0 | 0 |
| Hypertension | 0 | 0 |
| Diabetes mellitus | 0 | 0 |
| Other chronic diseases | 0 | 0 |

## Specimen collection

Venous blood samples were drawn in the fasting state for approximately 12 h and the samples were encoded for blind analysis. Peripheral blood mononuclear cells (PBMCs) were separated by Ficoll density-gradient centrifugation.

## Microarray analysis

The genome-wide analysis of mRNA and miRNA expression in DVT patients ($n = 6$) and control subjects ($n = 6$) were performed using Human mRNA and miRNA ($4 \times 180$ K, Design ID: 084410, $8 \times 60$ K, Design ID: 070156). Sample labeling, microarray hybridization and washing were performed by OE Biotech. Co., Ltd. (Shanghai, China) according to the manufacturer's standard protocols.

## Cell culture and transfection

293T cells were obtained from Procell Life Science & Technology Co., Ltd (Wuhan, China). 293T cells were transfected with miR-6132 mimics, inhibitor or respective negative control (GenePharma, Shanghai, China). The transfected cells were cultured in RPMI 1640 culture medium and harvested for 24 h. The sequences used in this study are listed in Table 2.

TABLE 2 The sequences of synthesized mimics, negative control (NC), inhibitor and inhibitor NC.

| Gene | Sequences (5′-3′) |
|---|---|
| hsa-miR-6132 mimics | Sense: AGCAGGGCUGGGGAUUGCA |
| | Antisense: CAAUCCCCAGCCCUGCUUU |
| NC | Sense: UUCUCCGAACGUGUCACGUTT |
| | Antisense: ACGUGACACGUUCGGAGAATT |
| hsa-miR-6132 inhibitor | UGCAAUCCCCAGCCCUGCU |
| Inhibitor NC | CAGUACUUUUGUGUAGUACAA |

TABLE 3 Quantitative real-time PCR (qRT-PCR) primers used in this study.

| Gene | Sequences (5′-3′) |
|---|---|
| GAPDH (homo sapiens) | F: GCACCGTCAAGGCTGAGAAC |
| | R: TGGTGAAGACGCCAGTGGA |
| Actin (mus musculus) | F: GGCTGTATTCCCCTCCATCG |
| | R: CCAGTTGGTAACAATGCCATGT |
| FOXP3 (homo sapiens) | F: CACTGCCCCTAGTCATGGTG |
| | R: CGTTGAGAGCTGGTGCATGA |
| Foxp3 (mus musculus) | F: ACTCGCATGTTCGCCTACTT |
| | R: TTGGCTCCTCTTCTTGCGAA |

## Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted using TRIzol Reagent (Invitrogen, Carlsbad, USA) following specification. Approximately 1 μg of RNA was reverse transcribed into cDNA, which was using SYBR Green (Invitrogen, Carlsbad, USA) for qRT-PCR analysis, and the relative mRNA levels were calculated using the $2^{-\Delta\Delta Ct}$ formula. The miRNA primers are designed and synthesized by RiboBio (Guangzhou, China). The PCR primer sequences are shown in Table 3.

## Dual-luciferase reporter assay

The FOXP3 wild-type (WT) and FOXP3 mutant (MUT) mRNA 3′UTR luciferase reporter vectors were constructed severally. 293T cells were co-transfected with WT or MUT luciferase reporter plasmids and the miR-6132 mimics or negative controls at an ultimate concentration of 100 nM. Luciferase activity was measured in cell lysates using a dual-luciferase reporter system 24 h after transfection.

## Flow cytometric analysis

Cells were resuspended in 1×PBS and stained with the following conjugated antibodies: CD4, FOXP3 (eBioscience, Ben Lomond, CA, USA) or their corresponding isotypic control for 30 min at 4°C in the dark. It was detected using the flow cytometer (Beckman Coulter, CytoFLEX, California, USA). All data were analyzed by using FlowJo _v10.8.1 (Treestar, USA).

## Western blotting

Samples were lysed in RIPA buffer with protease and phosphatase inhibitor (Solarbio, Beijing, China), and the proteins were subjected to SDS-PAGE and transferred onto nitrocellulose membranes. Then, the membranes were incubated with diluted primary antibodies followed by horseradish peroxidase HRP-conjugated secondary antibody. Antibodies against FOXP3 (1:1,000, ab215206, Abcam, Cambridge, USA) and GAPDH (1:10,000, ab181603, Abcam, Cambridge, USA) were used in this study.

## DVT mice model and treatment

C57BL/6J mice (8-week-old male) were purchased from Beijing HFK Bioscience Company (Beijing, China). Animal experiments were approved by the Institutional Animal Care and Use Committee of Shandong University of Traditional Chinese Medicine. The model of DVT mice was constructed according to the inferior vena cava (IVC) stenosis method, as described above (24).

The mice were stochastically falled into 4 groups (15/group): (1) DVT NC group; (2) DVT miR-6132 mimics group; (3) DVT INC group; (4) DVT miR-6132 inhibitor group.

For FOXP3 inhibitor experiments, mice were stochastically split into 3 groups (15/group): (1) DVT + INC group; (2) DVT miR-6132 inhibitor group; (3) DVT miR-6132 inhibitor + Epirubicin (FOXP3 inhibitor, MCE, USA) group. NC (5 nmol), miR-6132 mimics (5 nmol), INC (10 nmol), or miR-6132 inhibitor (10 nmol) were directly injected into the tail vein before undergoing the surgery, respectively. Epirubicin (0.3 μg/g) was injected into the tail vein before the mice underwent IVC stenosis and continued for three consecutive days (25). The fresh specimen section 2 mm below the IVC ligation site were sectioned for Hematoxylin and eosin (H&E) analysis.

## Murine doppler ultrasound

Mice were anesthetized using a mixture of isoflurane-oxygen for Vascular Doppler ultrasonography. Thrombus formation images were obtained using Small Animal Ultrasound Imaging System (VINNO6 LAB, VINNO, Suzhou, China).

## Enzyme-linked immunoassay (ELISA)

The protein expressions of endothelial nitric oxide synthase (eNOS) and endothelin-1 (ET-1) were detected by ELISA assay following the manufacturer's instructions with mice ELISA Kits, following the manufacturer's instructions. The absorbance of 450 and 630 nm was measured using a microplate reader.

## H&E

After embedding in paraffin, the tissues were cut into 4 μm-thick sections and stained with H&E following standard procedures. All histological images were observed via light microscopy (E100, Nikon, Japan).

## Statistical analyses

The results are expressed as the mean ± standard error, and the 2-tailed Student's *t* test or one-way ANOVA were used for comparisons. Statistical analysis was performed with SPSS 16.0 and GraphPad Prism 8.0 software. The diagnostic value was

evaluated by receiver operating characteristic (ROC) curve and correlations were analyzed by Pearson correlation. All experiments were representative of at least three times independently, unless otherwise noted. The values of $P < 0.05$ were considered statistically significant.

# Results

## FOXP3 was decreased under the condition of DVT

In order to demonstrate the physiological function and the pathogenesis of DVT, we performed an analysis of the biological processes involved in the downregulation of 1,866 differentially expressed genes ($P < 0.05$, FC > 1.5) using Gene Ontology (GO) terms. Among the top ten significantly enriched GO terms, we found differential enrichment of pathways involved in T cell activation (Figure 1A). Interestingly, we found FOXP3 was down-regulated in *T* cell activation pathway (Figure 1B). To identify the role of FOXP3 in the progression of DVT, we first examined the expression of FOXP3 and found that FOXP3 is relatively low expressed in DVT patients and DVT mice (Figures 1C,D,G). Meanwhile, the expression levels of IL-10 and TGF-β1 were also significantly reduced in DVT patients (Figures 1E,F).

## FOXP3 negatively correlated to miR-6132 in DVT patients

To further confirm the role of FOXP3 and miRNAs in DVT, we performed miRNA expression profiling. The mostly up-regulated 19 miRNAs were detected by at least 1.5-fold with $P < 0.01$ in DVT (Figure 2A). Here, we intersected the data from the TargetScan database and Chip, and displayed the possible interaction between miR-6132, miR-6785-5p, miR-574-5p, and FOXP3 (Figure 2B). Using amplified samples for validation, miR-6132 was robustly increased in DVT (Figures 2C–E). Next, miR-6132 was negatively correlated with FOXP3 (Figure 2F). With an area under the curve of 0.8989 (95% confidence interval: 0.8036–0.9942), ROC analysis indicated that miR-6132 was sensitive to distinguish DVT patients from healthy control subjects (Figure 2G). Together, our results suggested that miR-6132 and FOXP3 play important roles in DVT.

## miR-6132 attenuates the expression of FOXP3 *in vitro*

Database prediction discovered that miR-6132 shared binding sites with FOXP3 3′UTR (Figure 3A, Supplementary Figure S2A). Based on the dual-luciferase method, miR-6132 mimics significantly repressed luciferase activity of WT FOXP3 3′UTR, whereas did not affect MUT FOXP3 3′UTR (Figure 3B, Supplementary Figure S2B). In addition, we constructed the plasmids of WT 3′UTR and performed luciferase reporter assays to investigate whether miR-6132 might also target other factors closely related to DVT, such as IL-10 or TGF-β1. Notably, miR-6132 mimics could not inhibit the luciferase activity of IL-10 and TGF-β1 (Figures 4A,B). Taken together, our luciferase reporter analysis thus confirmed that FOXP3 is a direct target of miR-6132.

To further corroborate that FOXP3 inhibition was due to upregulation of miR-6132, we altered miR-6132 expression and determined the expression of FOXP3 in 293T cells. We observed that overexpression of miR-6132 using mimics (Figure 3C) correlated with downregulation of FOXP3 (Figures 3D,E), whereas inhibition of miR-6132 with miR-6132 inhibitor (Figure 3C) resulted in upregulation of FOXP3 (Figures 3D,E). Meanwhile, miR-6132 overexpression decreased the protein levels of IL-10 and TGF-β1 and miR-6132 knockdown promoted the protein levels of IL-10 and TGF-β1 (Figures 3F,G).

## miR-6132 inhibits the expression of FOXP3, which in turn affects the formation of DVT

To extend the functional role of miR-6132 in DVT formation, miR-6132 mimics, miR-6132 inhibitor or control vectors were injected via tail vein before DVT surgery. Our results revealed that miR-6132 was significantly increased in DVT miR-6132 mimics group (Figure 5E). Similarly, H&E staining, Doppler, the thrombus length and weight showed that thrombosis was aggravated in the DVT miR-6132 mimics group at 24 h after the ligation (Figures 5A–D). We next found that Foxp3 mRNA and protein decreased in spleen of mice treated with miR-6132 mimics (Figures 5F,G), indicating that miR-6132 could negatively regulate Foxp3 expression. In contrast, miR-6132 was significantly decreased in DVT miR-6132 inhibitor group (Figure 5E), and the thrombus length and weight greatly decreased in the DVT miR-6132 inhibitor group compared with those in DVT INC group (Figures 5A–D). Meanwhile, the expression of Foxp3 increased synchronously in mice treated with miR-6132 inhibitor (Figures 5F,G).

Moreover, expressions of IL-10, TGF-β1, and eNOS were significantly decreased, whereas ET-1 expression was increased in plasma of mice treated with miR-6132 mimics (Figures 5H,I, 6A,B). At the same time, expressions of IL-10, TGF-β1, and eNOS were obviously increased, whereas ET-1 expression was decreased in DVT miR-6132 inhibitor group (Figures 5H,I, 6A,B). These data indicated that inhibited miR-6132 could enhance FOXP3 expression and attenuate immune response, thereby alleviating endothelial dysfunction.

## Neutralization of FOXP3 promoted DVT formation *in vivo*

Compared with the sham groups, DVT groups showed obvious thrombosis, and IL-10 and TGF-β1 were down-regulated (Supplementary Figure S1A,B). To confirm whether Foxp3

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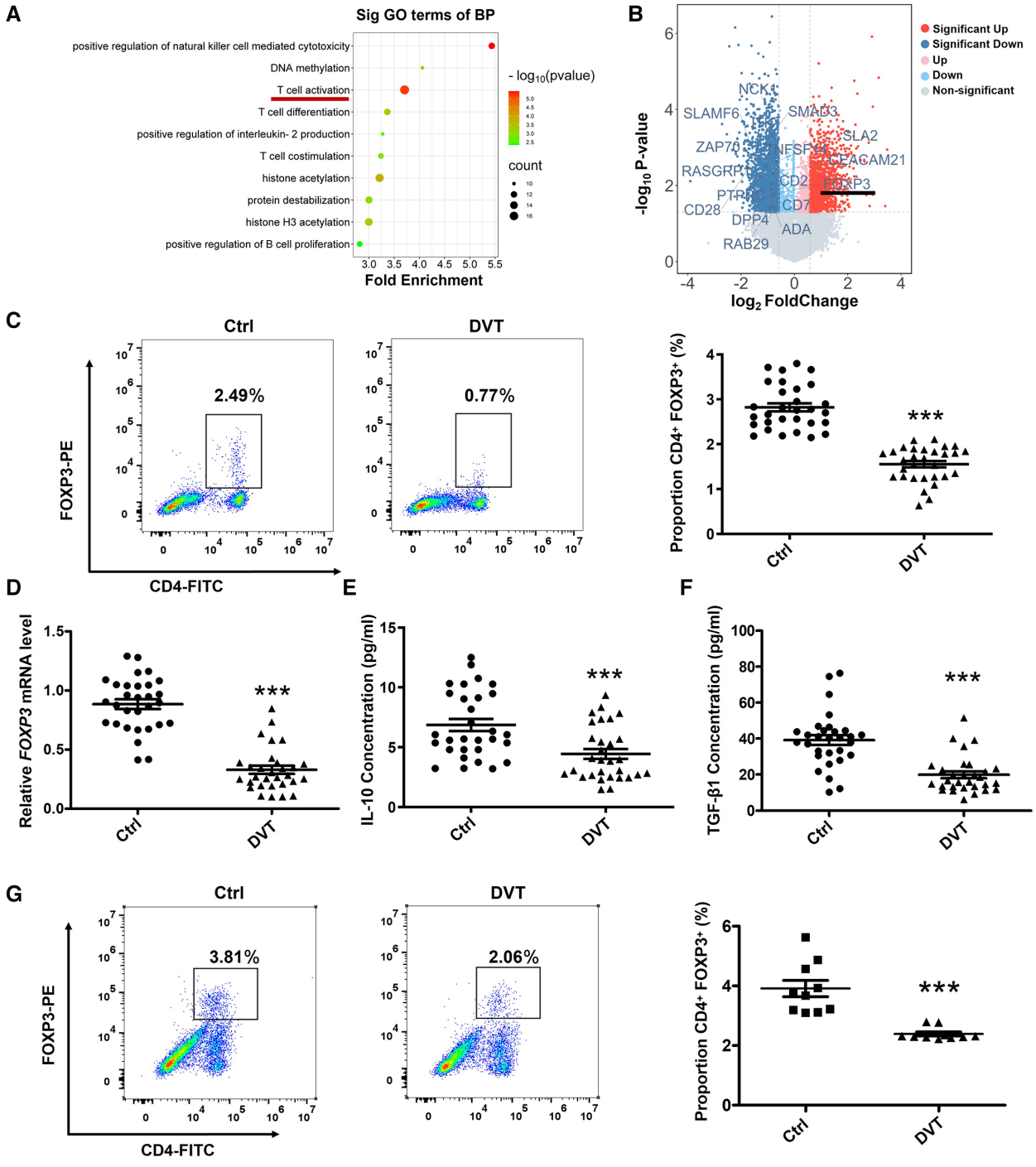


FIGURE 1
The expression of FOXP3, IL-10 and TGF-β1 are downregulated in DVT patients. (**A**) The top 10 gene ontology terms of biological process pathway enrichment analyses according to mRNA microarray analysis. (**B**) Volcano plot showing the profiling data of down-regulated mRNAs in the blood of six DVT patients compared with that of six controls determined by microarray analysis. Red indicates increased relative expression, while blue indicates decreased relative expression. (**C**,**D**) Relative expression levels of FOXP3 protein and mRNA in the peripheral blood mononuclear cells (PBMCs) from 30 DVT patients and 30 controls were determined by flow cytometry and quantitative real-time PCR (qRT-PCR). (**E**,**F**) IL-10 and TGF-β1 protein levels were determined by ELISA in plasma ($n = 30$). (**G**) FOXP3 protein levels were determined by flow cytometry in mice ($n = 10$). ***$P < 0.001$.

altered the DVT formation, we injected Epirubicin into mice to neutralize Foxp3 function. Notably, the size of thrombus was significantly increased (Figures 7A,B). Analogously, IL-10 and TGF-β1 was significantly decreased after injection of Epirubicin in plasma of DVT mice (Figures 7C,D). These data indicated that FOXP3 attenuated the formation of DVT.

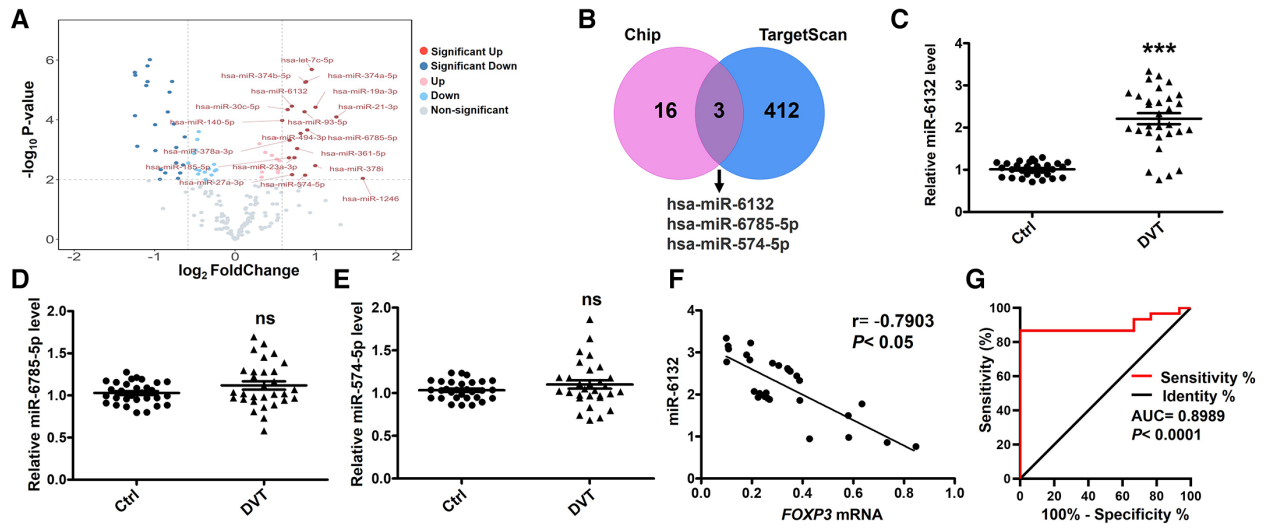


FIGURE 2
Negative correlation between the levels of miR-6132 and FOXP3 in patients with DVT. (A) Volcano plot showing the profiling data of up-regulated miRNAs in the blood of six DVT patients compared with that of six controls determined by microarray analysis. Red indicates increased relative expression, while blue indicates decreased relative expression. (B) The Venn diagram shows that miR-6132, miR-6785-5p and miR-574-5p were predicted by Targetscan and Chip. (C) Levels of miR-6132, miR-6785-5p and miR-574-5p in PBMCs from DVT patients ($n = 30$) compared with control subjects ($n = 30$) were measured by qRT-PCR. (D) The correlation between miR-6132 and FOXP3 was analyzed using Pearson correlation analysis ($n = 30$). (I) Diagnostic value of miR-6132 for DVT was evaluated by ROC curve. ***$P < 0.001$.

## Discussion

DVT is a process associated with abnormal epigenetic processes, and its pathogenesis involves complex events such as blood flow, blood components, endothelial injury, genetic predisposition, immunity, and inflammatory response (26–30). Despite increasing knowledge of risk factors, one third to one half of venous thromboembolism episodes do not have a clearly provoking cause (31, 32). The latest researches have proved that immune modulation may be involved in the occurrence and prevention of DVT (33). Here, the important role of Tregs in inducing and maintaining of immune homeostasis and tolerance,

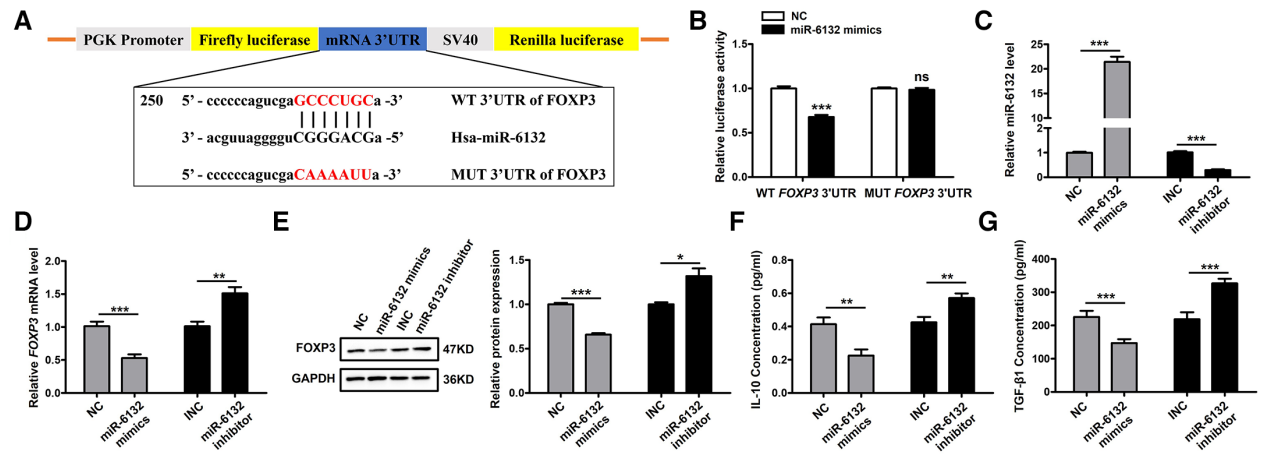


FIGURE 3
miR-6132 negatively regulates FOXP3 expression in 293T cells. (A) Potential miR-6132 binding sequence in *FOXP3* human mRNA 3′UTR. The sequence of the corresponding wild-type (WT) and mutant (MUT) constructs used in the luciferase reporter assay is also indicated. (B) The effect of negative control (NC) or miR-6132 mimics on luciferase activity transfected 293T cells expressing the WT or MUT 3′UTR of human FOXP3. (C) The expression level of miR-6132 after NC, miR-6132 mimics, inhibitor negative control (INC) and miR-6132 inhibitor transfection as detected by qRT-PCR. (D) The expression level of *FOXP3* mRNA after NC, miR-6132 mimics, INC and miR-6132 inhibitor transfection as detected by qRT-PCR. (E) The expression level of FOXP3 protein was detected by Western blot. (F,G) The expression levels of IL-10 and TGF-β1 protein were detected by ELISA. Results were quantified by Image J software. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

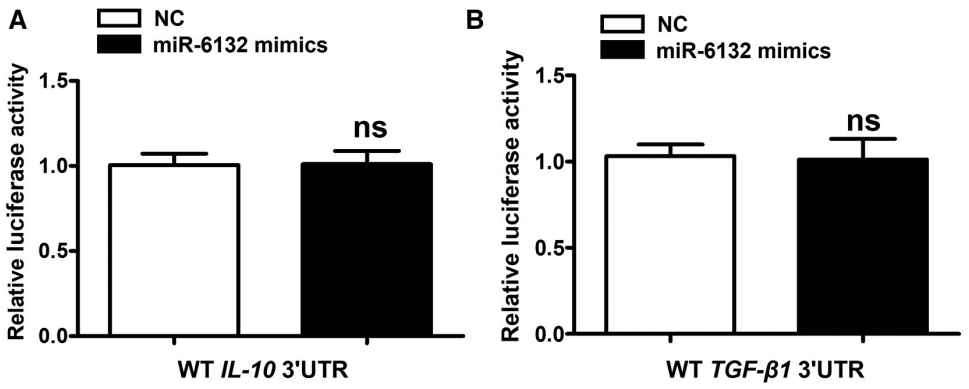


FIGURE 4
miR-6132 does not target IL-10, or TGF-β1 mRNA 3′UTR. (**A**,**B**) The luciferase activity was determined by co-transfecting the vectors (IL-10 3′UTR-WT or TGF-β1 3′UTR-WT) combined with negative control (NC) or miR-6132 mimics into 293T cells.

and Tregs dysfunction can lead to pathology and trigger abnormal immune responses (34). Similarly, Tregs play an indispensable role in many diseases such as autoimmune diseases, infectious diseases, organ transplantation and tumors (35–37). Evidence has demonstrated that the proportion of Tregs subgroups in DVT model rats was significantly reduced (8). Studies have reported FOXP3 has a strong regulatory effect on the function and plasticity of Treg cells, and is the main lineage master regulator

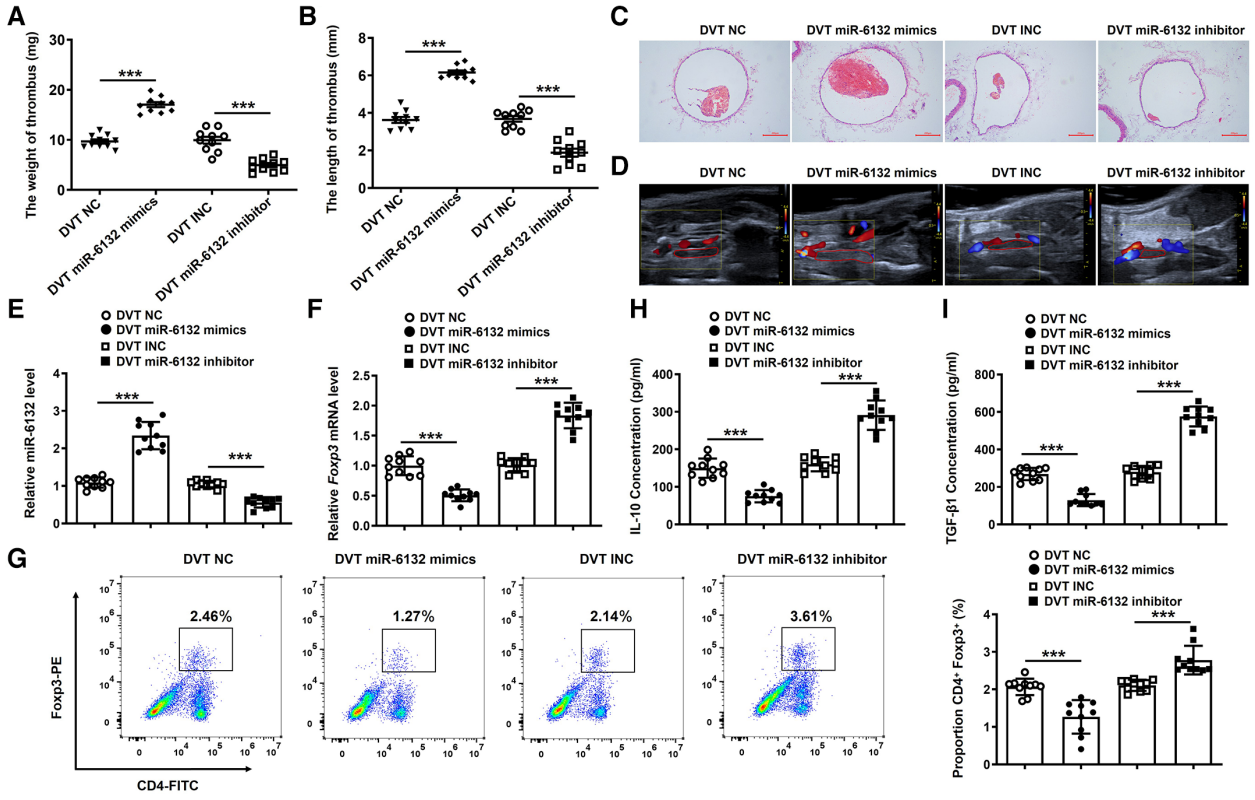


FIGURE 5
The regulatory effects of miR-6132 on the expression of FOXP3 in DVT formation. (**A**,**B**) The thrombosis weight and length in different groups ($n = 10$). (**C**,**D**) Representative images of thrombi in each group detected by H&E staining (magnification, ×100) and vascular ultrasound at 24 h post-operation. Scale bars = 200 μm. (**E**) Expression of miR-6132 was detected by qRT-PCR in spleen of each treatment group. (**F**,**G**) Foxp3 mRNA and protein levels were determined by qRT-PCR in spleen of each treatment group. (**H**,**I**) IL-10 and TGF-β1 protein levels were determined by ELISA in plasma of each treatment group. ***$P < 0.001$.

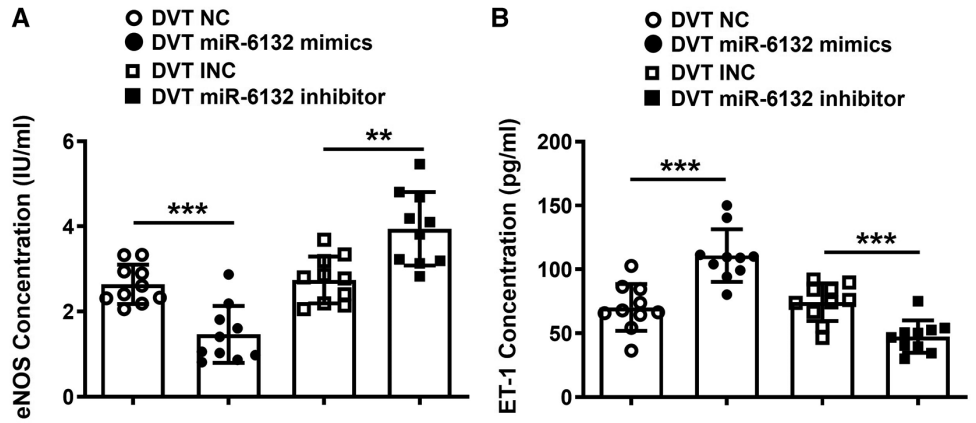


FIGURE 6
The regulatory effects of miR-6132 on the levels of eNOS and ET-1 were analyzed in plasma. (A,B) Expression of eNOS or ET-1 were determined by ELISA in plasma of each treatment group ($n = 10$). $^{**}P < 0.01$, $^{***}P < 0.001$.

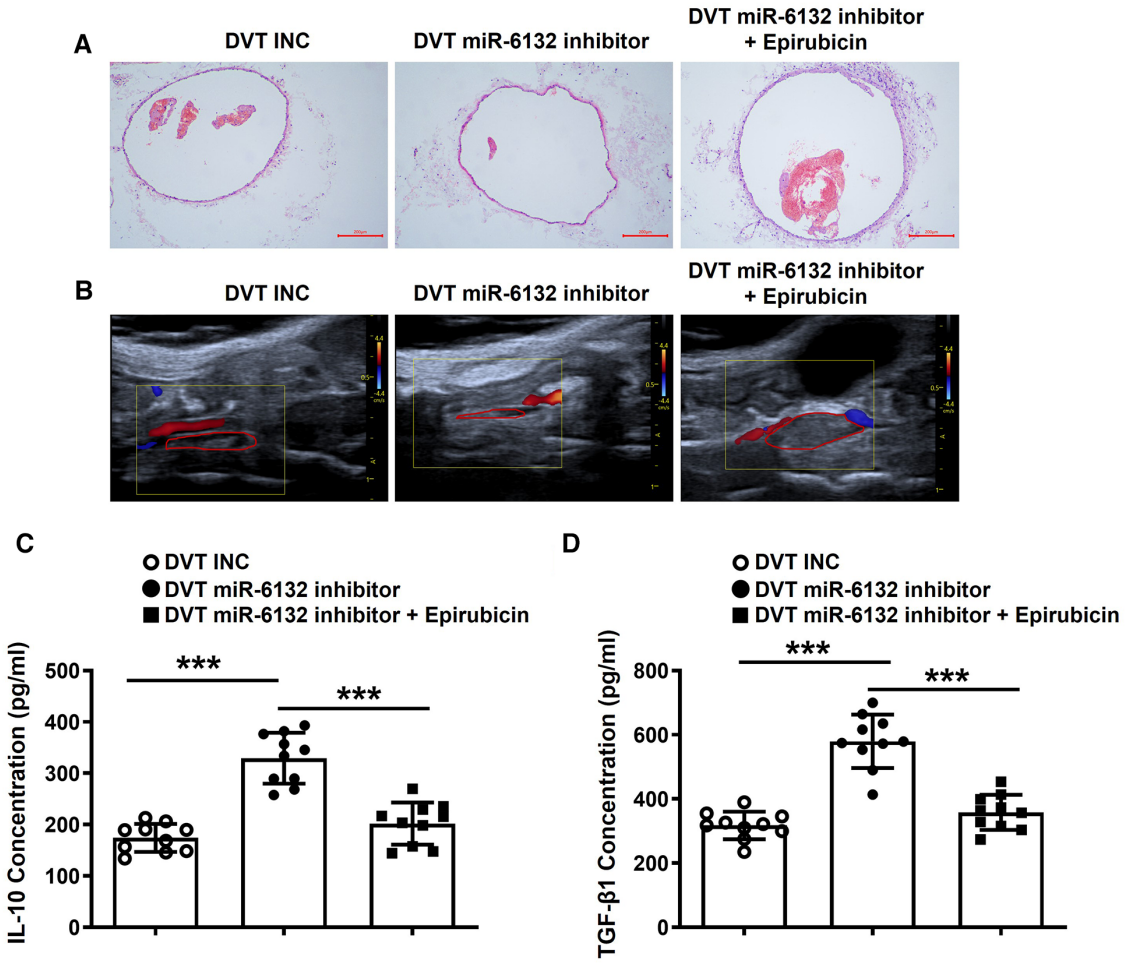


FIGURE 7
Neutralizing FOXP3 aggravated the formation of DVT *in vivo*. (A,B) Representative images of thrombi in each group detected by H&E staining and vascular ultrasound at 24 h post-operation (magnification, ×100). Scale bars = 200 μm. (C,D) IL-10 and TGF-β1 protein levels were determined by ELISA in plasma of each treatment group ($n = 10$). $^{***}P < 0.001$.

for Tregs development and inhibitory activity (38–40). Since FOXP3 is controlled by transcriptional and post-transcriptional mechanisms, inhibition of FOXP3 plays an important role in tissue damage. Analogously, the study provides insights into the role of FOXP3 in DVT through epigenetic modifications.

In the past, related studies have proved the importance of miRNAs in transcriptional regulation, and some miRNAs have been widely used as biomarkers and promising cancer therapeutic targets (41, 42). The pivotal role of miRNAs has become developing new diagnostic and therapeutic tools in in cardiovascular diseases, including DVT (43–45). In our previous research, it had been identified that miR-374b-5p can promote the development of DVT, and miR-338–5p inhibited the formation of DVT (46, 23). Furthermore, the expression of FOXP3 is regulated by several miRNAs such as miR-125b, miR-133a-3p, miR-34a, and miR-210. MiR-125b and miR-133a-3p are overexpressed, thereby reducing the expression of FOXP3 and further inducing autophagy in cancer (47–49). Down-regulation of miR-34a and miR-210 induces the expression of FOXP3, a major regulator that increases Tregs function, thus alleviating allergic diseases (50, 51). Further research is necessary to explore the mechanisms that the role of miRNA regulated FOXP3 in DVT. Through TargetScan database and Chip analysis, we found miRNAs that may have regulatory effects on FOXP3. We further expanded the sample for verification, and finally found that only miR-6132 may have a targeted regulatory relationship with FOXP3.

In many cases, microRNAs derived from the maternal gene, and the process was initiated by Drosha complex. Drosha's cleavage strictly depends on the secondary structure of the stem-loop base of pri-miRNA, as well as the sequence within the 125 nt range on the outer side of the stem-loop base. Therefore, the extremely specific nucleotides lying in the small region of sequence determined the generation of specific microRNAs. Given the large frequency of sequence variation across the species, some microRNA existed in human, but not mice, and hsa-miR-6132 is such a microRNA. To date, a mouse variant of hsa-miR-6132 has not annotated/determined. Although microRNAs are not conserved in mice, the functions of human microRNAs are usually validated in the mice model by acting on the same target gene (52, 53). In this study, we confirmed the regulatory function of hsa-miR-6132 on mouse Foxp3 with the same manner for human gene of FOXP3. To the best of our knowledge, our work provided that decreased miR-6132 increased FOXP3 expression and inhibited DVT formation (Figure 8). Thus, targeting miR-6132 may be a potentially effective strategy for DVT.

To gain a deeper understanding of the biological functions, Gene Ontology terms in Biological Process revealed that the markedly enriched pathways were the *T* cell activation signaling pathway. Here, our results showed that FOXP3 expression was obviously decreased in DVT. Coincidently, dual luciferase assay demonstrated the immediate bonding of miR-6132 to FOXP3 mRNA 3′UTR. Our pilot study showed that upregulation of miR-6132 could promote DVT formation by inhibiting FOXP3 expression. Based on the above findings, we further studied that inhibition of FOXP3 mRNA and protein levels by overexpression of miR-6132. Similarly, inhibition of miR-6132 could attenuate the formation of DVT by enhancing FOXP3 expression.

In conclusion, we first demonstrated that miR-6132 could contribute to DVT by suppressing the expression of FOXP3. The purpose of the study is to explore the biological role of miR-6132/FOXP3 axis, which will open up new potential marker for the diagnosis and treatment of DVT diseases in the future.

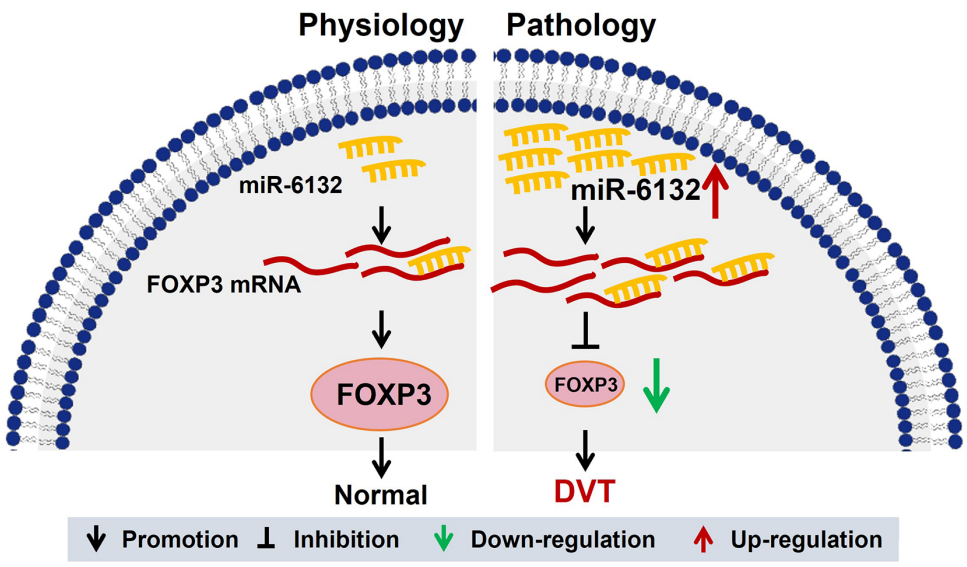


FIGURE 8
A graphical abstract showing the mechanism of miR-6132 and FOXP3 in DVT. The elevation of miR-6132 inhibited FOXP3 expression in DVT.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary Materials, further inquiries can be directed to the corresponding authors.

## Ethics statement

The studies involving humans were approved by the Ethics Committee of Shandong University of Traditional Chinese Medicine. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. The animal study was approved by the Ethics Committee of Shandong University of Traditional Chinese Medicine. The study was conducted in accordance with the local legislation and institutional requirements.

## Author contributions

YZ: Methodology, Project administration, Writing – original draft, Data curation, Investigation. ZZ: Conceptualization, Investigation, Writing – original draft. CC: Data curation, Supervision, Writing – original draft. GL: Project administration, Validation, Writing – original draft. NF: Data curation, Formal Analysis, Writing – original draft. HL: Resources, Validation, Writing – original draft. RW: Project administration, Writing – original draft. TZ: Methodology, Writing – original draft. LL: Data curation, Writing – original draft. BW: Funding acquisition, Supervision, Writing – review & editing. XL: Funding acquisition, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2024.1356286/full#supplementary-material

SUPPLEMENTARY FIGURE S1
The expression of IL-10 and TGF-β1 in DVT formation. (**A**) Representative images of thrombi in each group detected by H&E staining and vascular ultrasound at 24 h post-operation (magnification, ×100). Scale bars = 200 μm. (**B**) The levels of IL-10 and TGF-β1 were detected by ELISA in plasma. ***$P < 0.001$.

SUPPLEMENTARY FIGURE S2
Foxp3 was targeted and regulated by miR-6132. (**A**) Potential miR-6132 binding sequence in mouse Foxp3 mRNA 3′UTR. (**B**) The effect of negative control (NC) or miR-6132 mimics on luciferase activity expressing the wild-type (WT) and mutant (MUT) 3′UTR of mouse Foxp3. **$P < 0.01$.

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# Clinical characteristics of lower extremity deep vein thrombosis in young vs. middle-aged adults: a single-center retrospective study

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**Background:** The incidence of deep vein thrombosis (DVT) in the lower extremities is increasing in the younger population. However, there are fewer reported comparisons in the literature for lower extremity DVT.
**Methods:** Patients aged <40 years admitted with lower-extremity DVT between January 2018 and December 2023 were retrospectively analyzed and followed up for 1 year.
**Results:** A total of 61 patients were included in the study and divided into two groups: 33 patients over 30 years of age (middle-aged group) and 28 patients under 30 years of age (young group). A significant gender difference was observed, with a higher proportion of males in the young group compared to the middle-aged group ($P < 0.001$). Five patients in the young group were treated with anticoagulation alone, whereas all patients in the middle-aged group underwent endovascular therapy. A higher prevalence of inferior vena cava thrombosis in the young group compared to the middle-aged group (60.71% vs. 33.3%, $P = 0.032$). The proportion of iliac vein stenosis was significantly higher in the middle-aged groups than in the young group ($P = 0.002$). There was no statistically significant difference in venous function scores (Villalta and rVCSS) between the two groups during both the preoperative period and the postoperative follow-up ($P > 0.05$). The incidence of lower-extremity DVT post-thrombotic syndrome and thrombus recurrence was higher in the young group than in the middle-aged group at 1 year postoperatively (PTS: 78.57% vs. 33.3%, $P < 0.001$, and thrombus recurrence: 28.57% vs. 9.09%, $P < 0.05$). Univariate and multivariate analyses revealed that inferior vena cava thrombosis was an independent risk factor for severe DVT post-thrombotic syndrome and recurrent DVT ($P < 0.05$), whereas gender was an independent risk factor for recurrent DVT ($P < 0.05$).
**Conclusions:** This study suggests differences in the clinical characteristics and prognosis of lower-extremity DVT.

KEYWORDS

lower extremity deep vein thrombosis, young, PTS, thrombus recurrence, inferior vena cava thrombosis

## Introduction

The prevalence of lower extremity deep vein thrombosis (DVT) is primarily attributed to factors that affect blood flow, such as tumors, aging, hypercoagulability and prolonged immobilization (1, 2). These factors traditionally result in a lower incidence of DVT in younger individuals. However, in recent years, the prevalence of DVT has been on the

rise among the younger population in China (3). The formation of DVT can easily lead to post-thrombotic syndrome (PTS), a condition with later symptoms that significantly impact the patient's quality of life. This is particularly challenging for younger patients to accept. PTS, stemming from DVT in the lower extremities, inevitably diminishes the patient's quality of life, posing an unacceptable burden for the younger demographic. Recent literature suggests that DVT characteristics in young adults differ from those in older patients (4–6). However, detailed comparisons of DVT in China are scarce. Therefore, this study aims to conduct a retrospective analysis of 61 patients under the age of 40 admitted to our center. The analysis provides a preliminary exploration of DVT in the younger age group.

# Materials and methods

## Study design

A retrospective analysis was conducted on 61 patients under 40 years of age who were diagnosed with lower extremity deep vein thrombosis (DVT) from January 2018 to December 2023. The study was conducted at the Department of Vascular Surgery, Affiliated Hospital of Chengdu University of Traditional Chinese Medicine. Enrolled participants were confirmed to have venous thrombosis above the popliteal vein through lower extremity venous ultrasound. Only patients who were first-time diagnosed with lower extremity DVT and received anticoagulation therapy or underwent venography were included in the study. Exclusion criteria included patients who did not receive anticoagulation therapy or undergo venography.

## Treatment

All admitted patients received standard anticoagulation therapy using low molecular weight heparin sodium or rivaroxaban tablets (anticoagulant course >3 months). Additionally, all enrolled patients underwent lower extremity venography. For patients with acute iliofemoral vein thrombosis (onset within 14 days with markedly elevated D2 polymers), after communication with the patients and their families, the inferior vena cava filter is implanted and Angiojet thrombus is aspirated (7, 8). For cases with refractory thrombus, the surgeon will perform catheter contact thrombolysis (CDT) according to the situation. Balloon dilatation was performed for patients with iliac vein compression, and the decision to implant a stent was made by the surgeon as needed. Compression therapy was applied at the end of the procedure to alleviate lower limb swelling.

## Variables evaluated

In addition to basic information such as age, gender, and weight, the study collected indicators related to the increased risk of venous thrombosis, tumors, marital history, history of prolonged braking, and various biochemical indexes (hemoglobin, leukocytes, neutrophil counts, D2 polymorphisms, protein C, protein S, etc.). The location of lower extremity DVT was recorded by imaging, and the intraoperative Angiojet documented the thrombus location. The use of Angiojet thrombus aspiration catheter, CDT, balloon, and thrombus clearance were also indicated.

## Outcome

All patients underwent follow-up at the hospital and were contacted by telephone at 6 and 12 months postoperatively. Follow-up included lower extremity venous ultrasound, Villalta score assessment, and Revised venous clinical severity scores (rVCSS). A Villalta score exceeding 15 indicated severe lower extremity post-DVT syndrome.

## Statistical analysis

Normality of the quantitative data was assessed, and descriptive statistics were applied accordingly. To compare the young and middle-aged groups, *t*-tests were used for normally distributed data, Wilcoxon rank sum tests for non-normally distributed data, and chi-square tests/exact tests for categorical data. Risk factors for post-thrombotic syndrome (PTS) and thrombosis recurrence were analyzed using univariate and multivariate logistic regression. Statistical significance was determined at a significance level of $P < 0.05$. All analyses were performed using SAS statistical software (version 9.4, Cary, NC).

# Results

## Population characteristics, lesion characteristics, and surgical characteristics

This study included patients diagnosed with lower extremity deep vein thrombosis (DVT), 33 of whom were over 30 years old and 28 under 30. The proportion of males with lower extremity DVT was significantly higher in the younger population compared to the middle-aged and young adult population (78.57% vs. 33.3%, $P < 0.001$). The analysis of listed risk factors (Table 1) showed no history of tumors in either group. Family history was observed in only one case in each group. The young group had more case of recent hormone use, trauma, and history of deep vein thrombosis compared to the middle-aged group. On the other hand, the young group had fewer cases of history of braking, infections, and surgeries than in the middle-aged group. Table 2 show thathemoglobin levels were slightly higher in the young group ($4.29 \pm 0.67$ vs. $4.35 \pm 0.53$, $P = 0.705$) among the listed blood indices, Fibrinogen was elevated in the middle-aged group ($4.19 \pm 1.36$ vs. $4.84 \pm 2.62$, $P = 0.244$). Protein C deficiency was found in two cases, and protein S deficiency was found in

TABLE 1 Patient demographics.

| Variable | Total ($n = 61$) | Groupe | | Statistic | $P$ |
|---|---|---|---|---|---|
| | | <30 year ($n = 28$) | >30 year ($n = 33$) | | |
| Age, mean ± SD | 29.92 ± 7.01 | 23.36 ± 3.98 | 35.48 ± 3.04 | −13.468 | <.001 |
| BMI, mean ± SD | 23.37 ± 2.96 | 22.96 ± 3.71 | 23.71 ± 2.13 | 0.951 | 0.347 |
| Gender, *n* (%) | | | | 12.484 | <.001 |
| F | 28 (45.9) | 6 (21.43) | 22 (66.67) | | |
| M | 33 (54.1) | 22 (78.57) | 11 (33.33) | | |
| Hypertension, *n* (%) | 1 (1.64) | 0 (0.00) | 1 (3.03) | – | 1.000 |
| Diabetes, *n* (%) | 0 | 0 | 0 | – | – |
| Risk factors present % (*n*) | | | | | |
| Cancer | 0 | 0 | 0 | – | – |
| Marital history, *n* (%) | 13 (21.31) | 3 (10.71) | 10 (30.30) | 3.466 | 0.063 |
| Family history, *n* (%) | 2 (3.28) | 1 (3.57) | 1 (3.03) | – | 1.000 |
| Hormonal therapy, *n* (%) | 4 (6.56) | 3 (10.71) | 1 (3.03) | 0.475 | 0.491 |
| Recent surgery (2 months), *n* (%) | 25 (40.98) | 9 (32.14) | 16 (48.48) | 1.673 | 0.196 |
| Recent trauma (2 months), *n* (%) | 7 (11.48) | 4 (14.29) | 3 (9.09) | 0.053 | 0.817 |
| Immobilization, *n* (%) | 20 (32.79) | 6 (21.43) | 14 (42.42) | 3.030 | 0.082 |
| History of infection, *n* (%) | 10 (16.39) | 2 (7.14) | 8 (24.24) | 2.104 | 0.147 |
| May Thurner syndrome, *n* (%) | 1 (1.64) | 1 (3.57) | 0 (0.00) | – | 0.459 |
| DVT staging, *n* (%) | | | | – | 0.459 |
| Acute | 60 (98.36) | 27 (96.43) | 33 (100.00) | | |
| Chornic | 1 (1.64) | 1 (3.57) | 0 (0) | | |

TABLE 2 Hematology indicators.

| Variable | Total ($n = 61$) | Groupe | | Statistic | $P$ |
|---|---|---|---|---|---|
| | | <30 year ($n = 28$) | >30 year ($n = 33$) | | |
| White blood cell, $10^9$ L, Mean ± SD | 8.25 ± 1.93 | 8.59 ± 1.95 | 7.96 ± 1.90 | 1.278 | 0.206 |
| Erythrocyte, $10^{12}$ L, mean ± SD | 4.32 ± 0.59 | 4.29 ± 0.67 | 4.35 ± 0.53 | −0.381 | 0.705 |
| Hemoglobin, g/L, mean ± SD | 134.23 ± 18.55 | 138.82 ± 19.03 | 130.33 ± 17.48 | 1.815 | 0.075 |
| Fibrinogen, g/L, Mean ± SD | 4.54 ± 2.14 | 4.19 ± 1.36 | 4.84 ± 2.62 | −1.177 | 0.244 |
| D2 polymersug/ml, mean ± SD | 6.77 ± 5.66 | 6.90 ± 5.78 | 6.66 ± 5.66 | 0.165 | 0.869 |
| Protein C deficiency, *n* (%) | 2 (3.28) | 2 (7.14) | 0 (0.00) | – | 0.207 |
| Protein S deficiency, *n* (%) | 1 (1.64) | 1 (3.57) | 0 (0.00) | – | 0.459 |

one case in the young group, while none were found in the middle-aged group. As shown in Table 3, the analysis of the lesions showed a significantly higher incidence of inferior vena cava thrombosis among individuals under 30 years of age. Additionally, this group had a lower rate of iliac vein stenosis in this group (57.14% vs. 90.19%, $P = 0.002$).

TABLE 3 Summary of treatment characteristics.

| Variable | Total ($n = 61$) | Group | | Statistic | $P$ |
|---|---|---|---|---|---|
| | | <30 year ($n = 28$) | >30 year ($n = 33$) | | |
| Classification, *n* (%) | | | | | |
| Central | 21 (34.43) | 9 (32.14) | 12 (36.36) | 0.120 | 0.730 |
| Mixed | 37 (60.66) | 18 (64.29) | 19 (57.58) | 0.286 | 0.593 |
| Inferior vena cava thrombosis, *n* (%) | 28 (45.9) | 17 (60.71) | 11 (33.33) | 4.573 | 0.032 |
| Anticoagulant therapy alone, *n* (%) | 5 (8.2) | 5 (17.86) | 0 (0.00) | 4.265 | 0.039 |
| Angiojet, *n* (%) | 37 (60.66) | 17 (60.71) | 20 (60.61) | 0.000 | 0.993 |
| Catheter directed thrombolysis, *n* (%) | 23 (37.7) | 8 (28.57) | 15 (45.45) | 1.838 | 0.175 |
| Balloon, *n* (%) | 34 (55.74) | 15 (53.57) | 19 (57.58) | 0.098 | 0.754 |
| Stent, *n*, (%) | 20 (32.79) | 9 (32.14) | 11 (33.33) | 0.009 | 0.922 |
| Iliac vein stenosis or occlusion, *n* (%) | 46 (75.41) | 16 (57.14) | 30 (90.91) | 9.314 | 0.002 |
| Thrombus resolution, *n* (%) | 31 (50.82) | 16 (57.14) | 15 (45.45) | 0.828 | 0.363 |
| Elastic stockings, *n* (%) | 61 (100) | 28 (100) | 33 (100) | – | – |

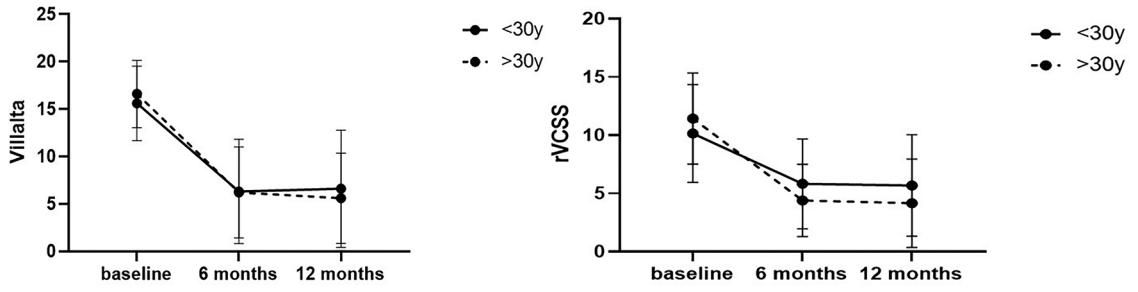


FIGURE 1
The score of vilata and revised venous clinical severity scores (rVCSS).

## Follow-up results

The mean Villalta scores improved at the 6-month and 12-month follow-ups for the young (baseline, 15.60; 6 months, 6.32; 12 months, 6.60; $P < .0001$), and middle age (baseline, 16.60; 6 months, 6.21; 12 months, 5.60; $P < .0001$) groups (Figure 1A). No difference in 6-month and 12-month Villalta score improvement was observed between the groups ($P > 0.05$). The mean rVCSS at baseline, 6 months, and 12 months was 10.14, 4.39, and 5.67, respectively, (6 months, $P < 0.001$; 12 months, $P < 0.001$; each follow up compared with baseline) for patients in the young group and 11.42, 5.82, and 0.15, respectively, ($P < .0001$ for each follow-up) for patients in the middle-aged group. Figure 1 illustrate variation in Villalta and rVCSS distribution between the groups. Improvement in 6-month and 12-month rVCSS was not significantly different between the groups. The young group had higher scores. A Villalta score exceeding 15 points indicatS severe PTS. The incidence of PTS was significantly higher in the young group than in the middle-aged group (78.57% vs. 33.3%, $P < 0.001$). Furthermore, the recurrence rate of lower extremity DVT was significantly higher in the youth group than in the middle-aged group (28.57% vs. 9.09%, $P < 0.05$, Table 4). The study conducted univariate and multivariate logistic regression analyses to determine the relationship between PTS and recurrence of lower extremity DVT. The results showed that inferior vena cava thrombosis was an independent risk factor for both PTS and recurrence of lower extremity DVT (Tables 5, 6). Additionally, Males were identified as an independent risk factor for the recurrence of lower extremity DVT (Table 6).

TABLE 4 Follow-up results of PTS and thrombosis recurrence after 12 months.

| Variable | Total ($n = 61$) | Groupe | | Statistic | $P$ |
|---|---|---|---|---|---|
| | | <30 year ($n = 28$) | >30 year ($n = 33$) | | |
| Severe PTS, $n$ (%) | 12 (19.67) | 8 (28.57) | 4 (12.12) | 2.594 | 0.107 |
| Thrombosis recurrence, $n$ (%) | 11 (18.03) | 8 (28.57) | 3 (9.09) | 3.889 | 0.049 |

## Discussion

The literature suggests that younger patients with lower-extremity DVT are not common in clinical practice. However, in recent years, the incidence of patients with lower-extremity DVT has increased compared with the past, and although the management of lower extremity DVT has gained a lot of diagnostic and therapeutic experience in the clinic, there is still a lack of more evidence-based medical evidence regarding the clinical characteristics of younger patients with DVTas well as their postoperative conditions. In this study, a preliminary attempt was made to gain a better understanding of the younger DVT patient population by retrospectively comparing the differences in lower extremity DVT between young and middle-aged patients.

The pathogenesis and recurrence of the first episode of DVT are multifactorial, depending on the severity and number of genetic and indirect factors (9). Venous thrombosis is caused by hypercoagulable state, venous stasis, and endothelial damage. The main conditions associated with these three pathological processes are tumors, trauma, immobility, and childbirth younger patients are less likely to develop DVT due to these underlying factors. Kuo DJ's reports that- prolonged repetitive squatting

TABLE 5 Univariate and multifactor logistic regression analysis of clinically relevant risk factors for PTS.

| Variables | Beta | S.E | $Z$ | OR (95% CI) | $P$ | aBeta | aS.E | aZ | aOR (95% CI) | aP |
|---|---|---|---|---|---|---|---|---|---|---|
| Age | −0.06 | 0.05 | −1.19 | 0.95 (0.86–1.04) | 0.235 | −0.03 | 0.05 | −0.55 | 0.97 (0.88–1.07) | 0.580 |
| Male | 1.14 | 0.73 | 1.57 | 3.12 (0.75–12.95) | 0.116 | 1.14 | 0.73 | 1.57 | 3.12 (0.75–12.95) | 0.116 |
| Anticoagulant therapy alone | 0.02 | 1.17 | 0.02 | 1.02 (0.10–10.08) | 0.985 | −1.42 | 1.39 | −1.03 | 0.24 (0.02–3.66) | 0.305 |
| Iliac vein stenosis or occlusion | −0.55 | 0.70 | −0.78 | 0.58 (0.15–2.29) | 0.436 | −0.68 | 0.90 | −0.76 | 0.51 (0.09–2.95) | 0.450 |
| Inferior vena cava thrombosis | 1.56 | 0.73 | 2.14 | 4.74 (1.14–19.74) | 0.033 | 1.65 | 0.77 | 2.15 | 5.22 (1.16–23.56) | 0.032 |

TABLE 6 Univariate and multifactor logistic regression analysis of clinically relevant risk factors for venous thrombosis recurrence.

| Variables | Beta | S.E | | OR (95% CI) | P | aBeta | aS.E | aZ | aOR (95% CI) | aP |
|---|---|---|---|---|---|---|---|---|---|---|
| Age | −0.07 | 0.05 | −1.46 | 0.93 (0.85–1.02) | 0.145 | −0.04 | 0.06 | −0.63 | 0.97 (0.87–1.08) | 0.526 |
| Male | 2.46 | 1.09 | 2.27 | 11.74 (1.40–98.74) | 0.023 | 2.46 | 1.09 | 2.27 | 11.74 (1.40–98.74) | 0.023 |
| Inferior vena cava thrombosis | 2.88 | 1.09 | 2.64 | 17.78 (2.10–150.38) | 0.008 | 3.11 | 1.15 | 2.71 | 22.34 (2.35–211.99) | 0.007 |
| Iliac vein stenosis or occlusion | −0.71 | 0.71 | −0.99 | 0.49 (0.12–2.00) | 0.323 | −1.10 | 1.03 | −1.07 | 0.33 (0.04–2.52) | 0.287 |
| Anticoagulant therapy alone | 0.14 | 1.17 | 0.12 | 1.15 (0.12–11.42) | 0.905 | −1.99 | 1.46 | −1.36 | 0.14 (0.01–2.40) | 0.174 |

position required by- baseball catchers may compress the blood vessels in the pelvic, inguinal, and popliteal regions, leading to the development of lower extremity DVT in younger baseball patients (5). In the Raymundo SRO report a patient with inferior vena cava malformation was found to have developed lower extremity DVT (10). Additionally, genetic factors such as abnormalities in blood proteins C and S and coagulation factor V can contribute to the development of lower extremity DVT. The study found elevated levels of protein C and S in patients under 30 years old, with less variation in other factors such as tumors. In the present study, it was found that males were more common among patients with DVT under 30 years of age, while females were more common among patients with DVT over 30 years of age. This difference was statistically significant.

The occurrence of post-thrombotic syndrome (PTS) and recurrent deep vein thrombosis (DVT) in young patients can have disastrous consequences. Long-term chronic venous hypertension can cause significant discomfort, and localized itching and ulceration of the lower extremities can affect the quality of life of patients. Therefore, in addition to preventing fatal pulmonary embolism in young patients with DVT, it is crucial to prevent recurrence of DVT and PTS in the lower extremities. According to the literature, severe post-thrombotic syndrome (PTS) occurs in 5%–10% of cases (11). However, this study found a significantly higher incidence of severe PTS in young and middle-aged patients. Additionally, patients under 30 years old with DVT had a significantly higher incidence of recurrent DVT and severe PTS within 1 year compared to young and middle-aged patients (Table 4). These findings warrant attention.

In 2017, an article published in the New England Journal of Medicine found no significant difference between anticoagulation alone and surgery in the management of lower extremity DVT with regards to the development of PTS in the later stages of the disease (12). However, the study's methodology suggests that different treatment modalities may impact the prognosis of lower extremity DVT (13–15). The discrepancy may be due to the previous study's prospective design, which introduced confounding factors, such as being conducted in the early stages of the disease. To ensure a meaningful comparison, it may be necessary to conduct a randomized controlled clinical trial, as there are several confounding factors to consider, such as the surgical procedure and type of thrombus. However, the study found no statistically significant difference in the rates of post-thrombotic syndrome (PTS) and lower extremity deep vein thrombosis (DVT) between the two treatment groups. It should be noted that the study had a small sample size and included age-specific patients, which limits the ability to draw conclusions about the effect of different treatment modalities on the occurrence of PTS and recurrence of thrombosis. Statin use has been shown (15) to reduce the incidence of PTS, and statins were not included in this study because the population consisted of young and middle-aged patients who were less likely to use lipid-lowering drugs.

Lower extremity DVT complicated by inferior vena cava thrombosis is rare and has a high mortality rate. Patients with untreated inferior vena cava thrombosis have a 90% risk of developing post-thrombotic syndrome (PTS), and up to 15% may develop severe PTS with chronic leg ulcers (16). Similarly, the incidence of inferior vena cava thrombosis was found to be as high as 60% in the young group in this study, with a significantly higher rate of PTS and recurrent thrombosis later in life than in the middle-aged group. Correlation analysis showed that the presence of inferior vena cava thrombosis in patients with lower-extremity DVT was an independent risk factor for PTS and recurrent venous thrombosis.

Regarding DVT recurrence, a prospective study by Dr. Legese Chelkeba et al. in an Ethiopian population showed that a history of previous DVT was an independent factor for DVT recurrence (2). In contrast, the patients enrolled in this study were all first diagnosed with thrombosis, which makes the two studies not comparable considering the age and ethnicity of the cohort in this study population. However, we found that male sex was an independent risk factor for recurrence in patients with lower-extremity DVT. This has been less reported in previous literature and further studies are needed to confirm this.

However, this study is a small retrospective analysis of rejuvenated lower extremity DVT patients, comparing those over 30 years old with those under 30 years old. While there are differences between the two age groups, the age difference is not significant enough to highlight the characteristics of rejuvenated DVT in certain factors. Therefore, multicenter prospective studies or randomized clinical controlled trials are needed to investigate the rejuvenation of deep vein thrombosis (DVT) in the lower extremities. This will increase awareness of young DVT and provide a more evidence-based approach to treatment at a later stage.

## Conclusion

Our study examines the unique characteristics of deep vein thrombosis (DVT) in young adults vs. middle-aged individuals. We found that young adults have higher rates of post-thrombotic syndrome (PTS) and recurrent DVT. We also identified inferior vena cava thrombosis as a potential independent risk factor for both PTS and DVT. Furthermore, gender was found to be a

significant risk factor for recurrent thrombosis. These findings emphasize the necessity of customized approaches to comprehend and manage DVT in young adults. It is important to focus on factors that may increase the risk of PTS and recurrence.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

## Ethics statement

The requirement of ethical approval was waived by the Medical Ethics Committee of Chengdu University of Traditional Chinese Medicine Hospital for the studies involving humans because this is a retrospective analysis. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

## Author contributions

YZ: Data curation, Investigation, Writing – original draft. HW: Data curation, Supervision, Writing – original draft. YL: Formal Analysis, Supervision, Writing – review & editing. QW: Conceptualization, Data curation, Validation, Writing – review & editing. CH: Conceptualization, Methodology, Software, Supervision, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



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# Blood urea nitrogen to creatinine ratio is associated with in-hospital mortality in critically ill patients with venous thromboembolism: a retrospective cohort study

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**Background:** The relationship between the blood urea nitrogen to creatinine ratio (BCR) and the risk of in-hospital mortality among intensive care unit (ICU) patients diagnosed with venous thromboembolism (VTE) remains unclear. This study aimed to assess the relationship between BCR upon admission to the ICU and in-hospital mortality in critically ill patients with VTE.

**Methods:** This retrospective cohort study included patients diagnosed with VTE from the Medical Information Mart for Intensive Care IV (MIMIC-IV) database. The primary endpoint was in-hospital mortality. Univariate and multivariate logistic regression analyses were conducted to evaluate the prognostic significance of the BCR. Receiver operating characteristic (ROC) curve analysis was utilized to determine the optimal cut-off value of BCR. Additionally, survival analysis using a Kaplan–Meier curve was performed.

**Results:** A total of 2,560 patients were included, with a median age of 64.5 years, and 55.5% were male. Overall, the in-hospital mortality rate was 14.6%. The optimal cut-off value of the BCR for predicting in-hospital mortality in critically ill VTE patients was 26.84. The rate of in-hospital mortality among patients categorized in the high BCR group was significantly higher compared to those in the low BCR group (22.6% vs. 12.2%, $P < 0.001$). The multivariable logistic regression analysis results indicated that, even after accounting for potential confounding factors, patients with elevated BCR demonstrated a notably increased in-hospital mortality rate compared to those with lower BCR levels (all $P < 0.05$), regardless of the model used. Patients in the high BCR group exhibited a 77.77% higher risk of in-hospital mortality than those in the low BCR group [hazard ratio (HR): 1.7777; 95% CI: 1.4016–2.2547].

**Conclusion:** An elevated BCR level was independently linked with an increased risk of in-hospital mortality among critically ill patients diagnosed with VTE. Given its widespread availability and ease of measurement, BCR could be a valuable tool for risk stratification and prognostic prediction in VTE patients.

KEYWORDS

venous thromboembolism, blood urea nitrogen to creatinine ratio, intensive care unit, in-hospital mortality, critically ill

# 1 Introduction

Venous thromboembolism (VTE), which includes deep vein thrombosis (DVT) and pulmonary embolism (PE), is a potentially preventable complication in critically ill patients (1). It is often associated with morbidity, mortality, and financial burdens, making it one of the significant contributors to the global burden of disease (1–3). The risk of VTE is significantly higher in critically ill patients compared to those hospitalized for other medical conditions (2). Although therapeutic advancements for VTE have resulted in improved patient outcomes in recent years, mortality rates continue to be higher, especially among critically ill patients (4–7). Prior research has identified several prognostic factors linked to in-hospital mortality in VTE patients, including age, preexisting comorbidities, VTE type, severity of illness, time to diagnosis and treatment, anticoagulation type, bleeding complications, and various laboratory parameters (8–10). Given the risk of VTE, identifying non-invasive and inexpensive tests for prompt recognition of high-risk patients with an increased mortality risk is key for improving patient care and reducing the impact of this potentially fatal condition.

Blood urea nitrogen (BUN) and creatinine are nitrogenous terminal products that indicate human renal function. Nevertheless, growing evidence suggests a possible correlation between these indicators and neurohormonal activity (11, 12). Dysregulated cardiorenal function and increased neurohormonal activation may contribute to higher levels of BUN and creatinine, which have been associated with increased mortality in several diseases (12–14). Many factors, such as medications, protein intake, muscle mass, and dehydration, influence BUN and creatinine levels (15, 16). Therefore, the BUN to creatinine ratio (BCR) is more valuable than either BUN or creatinine alone, as it is less prone to fluctuations and better reflects kidney function. The BCR is a useful predictor of outcomes in various diseases, and a high BCR is linked to increasing in-hospital mortality in critically ill patients, including those with septic shock (17), acute myocardial infarction (18), cerebral infarction (19), acute respiratory distress syndrome (20), cardiogenic shock (21), and COVID-19 (22). In patients with VTE, cardiorenal function and neurohumoral regulation are impaired due to various factors such as systemic hypoxia, activation of chemoreflex, hypercapnia, and inflammatory state, leading to elevated BUN and creatinine levels (23–27).

Despite the evidence mentioned above, to the best of our knowledge, no prior research has investigated the potential association between BCR and mortality rates in critically ill patients diagnosed with VTE. Therefore, utilizing the Medical Information Mart for Intensive Care-IV (MIMIC-IV) database (28), we hypothesized that an elevated BUN/creatinine ratio could increase the risk of in-hospital mortality among ICU patients with VTE.

# 2 Material and methods

## 2.1 Data source

All data used in this retrospective cohort study were extracted from the Medical Information Mart for Intensive Care-IV (MIMIC-IV) version 1.0, which includes electronic health records of adult patients admitted to the intensive care unit at Beth Israel Deaconess Medical Center in Boston, Massachusetts, between 2008 and 2019. Access to the database is granted to individuals who have completed the "Protecting Human Research Participants" training. For this study, author AP obtained the necessary certification and extracted the relevant data from the database (certification number: 61239194). Our research complied with the ethical principles outlined in the Helsinki Declaration. This study was approved by the Institutional Review Boards of Beth Israel Deaconess Medical Center and the Massachusetts Institute of Technology (Cambridge, MA, USA). Informed consent was not required as patient health information in this database was anonymized (28).

## 2.2 Study population

The diagnosis of VTE was based on the International Classification of Diseases, Ninth Revision (ICD-9) code, and the Tenth Revision (ICD-10) code. The ICD codes used for identifying patients with VTE are presented in Supplementary Table S1. Patients aged 18 years or older diagnosed with VTE were included in this study. Patients with multiple ICU admissions, those under 18 years old, individuals with an ICU stay of less than 24 h, and patients with missing data on BUN or creatinine were excluded from this study.

## 2.3 Data extraction

The Structured Query Language (SQL) was utilized to extract data using script codes obtained from the GitHub repository (https://github.com/MIT-LCP/mimic-iv). The following variables were collected: age, gender, length of stay (LOS) at the hospital, length of stay at the ICU, hospital death sign, Charlson comorbidity index (CCI), Simplified Acute Physiology Score II (SAPS II), laboratory tests on the day one of admission including hemoglobin, white blood cells (WBC), red blood cells (RBC), platelets, glucose, mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), hematocrit, blood urea nitrogen (BUN), creatinine, bicarbonate, international normalized ratio (INR), prothrombin time (PT), and partial prothrombin time (PTT). The extracted preexisting comorbidities included hypertension, diabetes, congestive heart failure, coronary artery disease, renal disease, severe liver disease, obesity, malignant cancer, cerebrovascular disease, and chronic obstructive pulmonary disease (COPD). The extracted data also encompasses vital signs, such as heart rate, respiratory rate, mean arterial pressure (MAP), and peripheral oxygen saturation (SpO2). Information on whether patients required mechanical ventilation, received renal replacement therapy (RRT), or used diuretics was also extracted. The average value was used if a variable was assessed multiple times on the first day of admission. To mitigate potential bias, variables with missing values >20% were

excluded. For variables with less than 20% missing values, the random forest imputation method from the missForest package in R software was used for imputation (29).

## 2.4 Outcomes

The primary outcome in the present study was in-hospital mortality. Secondary outcomes included length of ICU stay and length of hospital stay.

## 2.5 Statistical analysis

The normality of the variables was assessed using the Kolmogorov-Smirnov test. Results were presented as mean ± standard deviation (SD) for normally distributed data, and the independent sample *t*-test was employed for comparison between the groups. Conversely, variables were described as median with interquartile range (IQR) for non-normally distributed data, and the Mann–Whitney test was used for comparisons. Categorical variables were presented as total numbers and percentages, and the chi-square test or Fisher's exact test was utilized for analyses.

The characteristics of the patients included in the study were compared between the survival group and the deceased group. The optimal threshold values for BCR associated with in-hospital mortality were determined using the maximum Youden index through receiver operating characteristic (ROC) curve analysis. Subsequently, all patients with VTE were divided into two groups based on these cut-off values: the high BCR group and the low BCR group. Binomial logistic regression analysis was conducted to evaluate the influence of BCR on in-hospital mortality among patients with VTE. Variables with a *p*-value <0.1 in the univariate analysis and potential confounders identified through clinical expertise were included in the multivariate analysis. The crude model did not incorporate any adjustments to variables. In the multivariable analysis, three models (Model I, Model II, Model III) were developed to examine the association between BCR and in-hospital mortality. Survival curves were constructed utilizing the Kaplan-Meier method, with the log-rank test employed to compare survival rates between the high BCR and low BCR groups. All statistical analyses were conducted using SPSS version 26.0 and MedCalc version 19.6 software. A *p*-value of less than 0.05 was considered statistically significant.

# 3 Results

## 3.1 Baseline characteristics of the participants

This study included a total of 2,560 critically ill patients diagnosed with VTE. The flowchart for patient screening is depicted in Figure 1. The median age of the participants with VTE was 64.5 years, and 55.5% were male. The in-hospital mortality rate was 14.6% (373/2,560). Table 1 summarizes the baseline characteristics of both the survival and deceased groups. Compared with patients in the survival group, the patients in the death group tended to be older. They had a higher proportion of diabetes, congestive heart failure, renal disease, severe liver disease, obesity, and cancer (all $P < 0.05$). Individuals in the deceased group exhibited elevated levels of creatinine, white blood cells (WBC), red cell distribution width (RDW), heart rate, and respiratory rate compared to those in the survival group (all $p < 0.05$). Conversely, they demonstrated lower mean arterial pressure, hemoglobin, red blood cell (RBC) count, platelet count, mean corpuscular hemoglobin concentration (MCHC), hematocrit, and bicarbonate levels (all $p < 0.05$). The CCI and SAPS II scores were higher in the deceased group, whereas the length of hospital stay was shorter compared to the survival group (all $p < 0.05$). Moreover, patients in the death group were more likely to receive RRT and diuretics, and they more frequently required mechanical ventilation during their hospital stay compared to the survival group (all $p < 0.05$). We found that patients in the death group had significantly higher levels of BUN, creatinine, and BCR when compared with the survival group (Table 1).

## 3.2 The prognostic significance of BCR

The ROC curve was generated for BCR to predict in-hospital mortality in critically ill patients with VTE, and the area under the ROC curve was 0.587 (95% CI: 0.568–0.607, $P < 0.001$) (Figure 2). The optimal cut-off value of BCR to predict survival status was 26.84, with a sensitivity of 35.93% and a specificity of 79.06%. Using this cut-off value, patients were categorized into two groups: the low BCR group (≤26.84, $n = 1{,}966$) and the high BCR group (>26.84, $n = 594$). Table 2 displays the baseline characteristics of the low BCR and high BCR groups. In comparison with patients in the low BCR group, those in the high BCR group exhibited a significantly higher in-hospital mortality rate (22.6% vs. 12.2%, $P < 0.001$). Patients with a higher BCR (>26.84) were more likely to have comorbid diseases, including congestive heart failure, coronary artery disease, severe liver disease, cancer, and COPD, as well as higher CCI, SAPS II scores, hemoglobin levels, platelet counts, RBC counts, MCHC, RDW, hematocrit, PT, and INR levels compared to those with a low BCR (≤26.84) (all $P < 0.05$). Additionally, individuals with a BCR >26.84 were prone to experiencing prolonged hospital and ICU stays ($p < 0.05$). They also exhibited a higher likelihood of receiving diuretic therapy, mechanical ventilation, and renal replacement therapy compared to those with a low BCR ≤26.84 (all $P < 0.05$) (Table 2).

## 3.3 Association between BCR and in-hospital mortality in patients with VTE

The results of the univariable logistic regression analysis are presented in Table 3. Additionally, Table 4 illustrates the unadjusted and multivariable-adjusted correlations between BCR

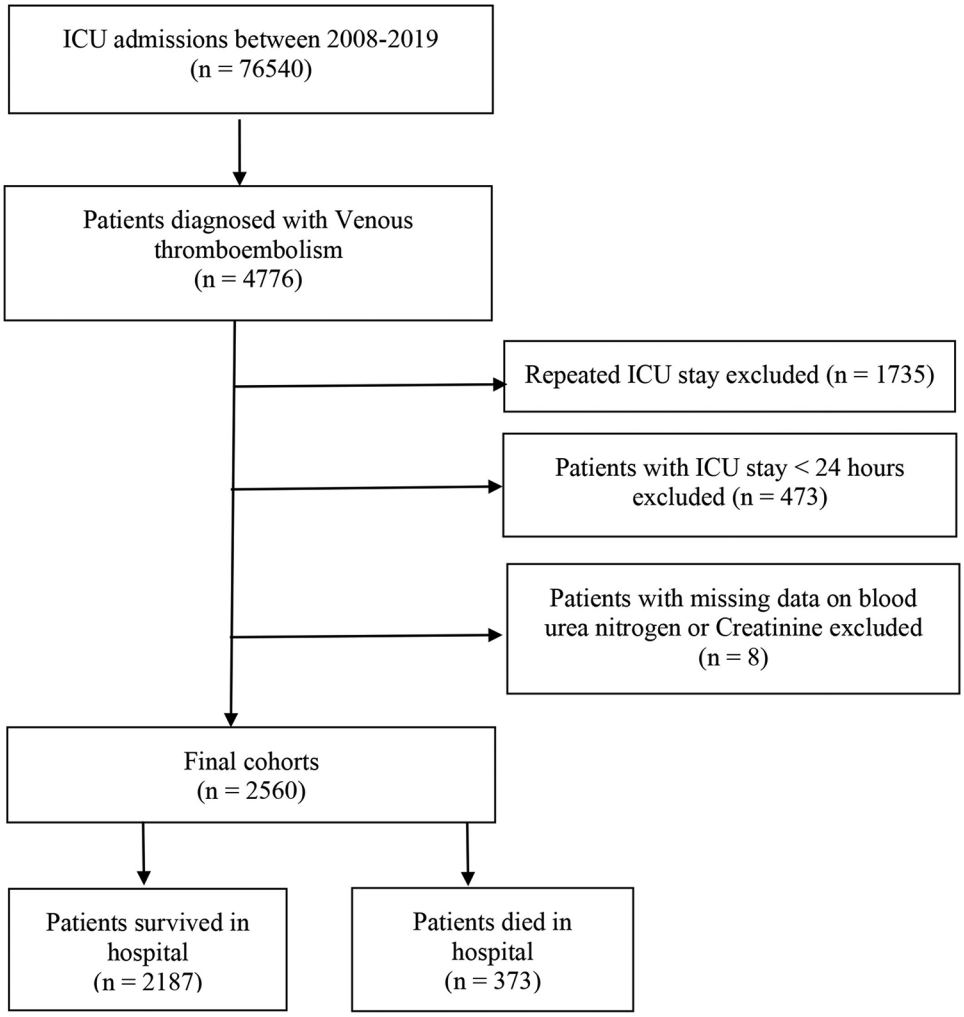


FIGURE 1
Flowchart for selecting analyzed participants.

and in-hospital mortality. The logistic regression model was employed to evaluate the impact of exposure variables on the outcome measures while adjusting for covariates. The crude model was not adjusted. In Model I, age and gender were incorporated as covariates to account for potential confounders. We adjusted for 11 variables in Model II, including hypertension, diabetes, congestive heart failure, coronary artery disease, renal disease, severe liver disease, obesity, malignant cancer, mechanical ventilation, diuretic use, and renal replacement therapy. Model III was adjusted for 18 variables, including CCI, SAPS II Score, hemoglobin, WBC, RBC, platelets, glucose, MCHC, RDW, hematocrit, bicarbonate, INR, PT, APTT, HR, MAP, RR, and SpO2. A statistically significant positive association was observed between the BCR (a continuous variable) and in-hospital mortality across all models: Crude Model: OR = 1.029, 95% CI: 1.020–1.038, $P < 0.001$; Model I: OR = 1.024, 95% CI: 1.014–1.033, $P < 0.001$; Model II: OR = 1.032, 95% CI: 1.023–1.042, $P < 0.001$; Model III: OR = 1.016, 95% CI: 1.006–1.026, $P = 0.002$). Additionally, compared to the low BCR (≤26.84) group, in-hospital mortality was significantly higher in the high BCR (>26.84) group across different models: Crude Model (OR = 2.105, 95% CI: 1.664–2.663, $P < 0.001$); Model I (OR = 1.874, 95% CI: 1.874–2.381, $P < 0.001$); Model II (OR = 2.091, 95% CI: 1.626–2.688, $P < 0.001$); and Model III (OR = 1.420, 95% CI: 1.086–1.857, $P = 0.010$) (Table 4).

## 3.4 Kaplan–Meier survival analysis of the cohort by BCR groups

A Kaplan-Meier curve was generated to demonstrate the survival outcomes of patients with VTE in both the high BCR and low BCR groups (Figure 3). The median survival time for the low BCR group was 86.976 days (95% CI: 77.704–377.026), whereas for the high BCR group, it was 45.477 days (95% CI: 38.876–80.425). This difference was statistically significant (log-rank test, $P < 0.0001$). In comparison to the low BCR group, the

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TABLE 1 Demographic and clinical characteristics of patients with venous thromboembolism.

| Characteristics | Total ($n = 2,560$) | Survival ($n = 2,187$) | Death ($n = 373$) | $p$-value |
|---|---|---|---|---|
| Age, year | 64.5 (53–75.5) | 64 (52–74.5) | 69 (60–79) | <0.001 |
| Gender, male | 1,420 (55.5%) | 1,226 (56.1) | 194 (52) | 0.146 |
| CCI | 6 (4–8) | 5 (3–7) | 7 (6–10) | <0.001 |
| SAPS II Score | 36 (27–46) | 34 (25–43) | 49 (38–61) | <0.001 |
| Laboratory tests | | | | <0.001 |
| Hemoglobin, g/dl | 10.7 (9.2–12.4) | 10.8 (9.4–12.5) | 10 (8.6–11.6) | <0.001 |
| WBC, 109/L | 11.2 (8.3–15.1) | 11.1 (8.2–14.6) | 12.7 (8.7–18.4) | <0.001 |
| RBC, 109/L | 3.6 (3.1–4.2) | 3.6 (3.2–4.2) | 3.4 (2.9–3.9) | <0.001 |
| Platelets, 109/L | 192 (133–264) | 194.5 (137–264.5) | 170 (100.3–261.7) | <0.001 |
| Glucose, mg/dl | 129 (109–160.5) | 127.5 (108.5–159) | 137.5 (111.5–172.5) | <0.001 |
| MCHC, g/dl | 33.1 (32.1–34.1) | 33.1 (32.2–34.2) | 32.7 (31.6–33.7) | <0.001 |
| RDW, % | 14.8 (13.7–16.5) | 14.6 (13.6–16.2) | 16 (14.6–17.8) | <0.001 |
| Hematocrit, % | 32.2 (28–37) | 32.5 (28.3–37.2) | 30.8 (26.6–35.1) | <0.001 |
| BUN, mg/dl | 19 (13–30) | 18 (12.5–28) | 28 (18–43) | <0.001 |
| Creatinine, ng/dl | 1 (0.7–1.4) | 0.9 (0.7–1.3) | 1.2 (0.8–2) | 0.040 |
| BUN/Cr ratio | 19.1 (14.3–26.1) | 18.7 (14.1–25.1) | 22 (15.5–30.8) | <0.001 |
| Bicarbonate, mEq/L | 23 (20–25.5) | 23 (20.5–25.5) | 21.5 (18–24) | <0.001 |
| Coagulation tests | | | | <0.001 |
| INR | 1.3 (1.2–1.5) | 1.3 (1.2–1.5) | 1.4 (1.2–1.9) | <0.001 |
| PT | 14.4 (12.9–16.8) | 14.3 (12.8–16.4) | 15.8 (13.6–20.3) | <0.001 |
| PTT | 34.4 (28.8–59) | 35.6 (28.5–57.7) | 41.1 (30.2–67.4) | <0.001 |
| Comorbidities | | | | |
| Hypertension | 1,014 (39.6) | 881 (40.3) | 133 (35.7) | 0.091 |
| Diabetes | 623 (24.3) | 514 (23.5) | 109 (29.2) | 0.017 |
| Congestive heart failure | 593 (23.2) | 482 (22) | 111 (29.8) | 0.001 |
| Coronary artery disease | 464 (18.1) | 383 (17.5) | 81 (21.7) | 0.051 |
| Renal disease | 385 (15) | 304 (13.9) | 81 (21.7) | <0.001 |
| Severe liver disease | 246 (9.6) | 184 (8.4) | 62 (16.6) | <0.001 |
| Obesity | 334 (13) | 299 (13.7) | 35 (9.4) | 0.023 |
| Malignant cancer | 586 (22.9) | 445 (20.3) | 141 (37.8) | 0.000 |
| Cerebrovascular disease | 392 (15.3) | 325 (14.9) | 67 (18) | 0.124 |
| COPD | 649 (25.4) | 544 (24.9) | 105 (28.2) | 0.179 |
| Monitoring parameters | | | | |
| Heart rate, Bpm | 89 (78–102) | 89 (77–101) | 93 (81–104) | <0.001 |
| MAP, mmHg | 78 (71–86) | 78 (72–87) | 75 (68–82) | <0.001 |
| RR, breaths/minutes | 19 (17–23) | 19 (17–22) | 21 (18–24) | <0.001 |
| SpO2,% | 97 (95–98) | 97 (96–98) | 97 (95–98) | 0.005 |
| Intervention | | | | |
| Mechanical ventilation | 991 (38.5) | 804 (36.8) | 187 (50.1) | <0.001 |
| Diuretic use | 382 (14.9) | 305 (13.9) | 77 (20.6) | 0.001 |
| RRT | 205 (8) | 131 (6) | 74 (19.8) | <0.001 |
| Outcomes | | | | |
| LOS hospital, day | 12.2 (6.9–20.9) | 12.4 (7–20.9) | 11.8 (5.3–20.5) | 0.018 |
| LOS ICU, day | 3.2 (1.9–6.9) | 3 (1.9–6.3) | 4.7 (2.4–10.1) | 0.000 |

Values are expressed as the median (Interquartile range) or *n* (%). CCI, Charlson Comorbidity Index; SAPS, the simplified acute physiology score; WBC, white blood cells; RBC, red blood cell; MCHC, mean corpuscular hemoglobin concentration; RDW, red blood cell distribution width; BUN, blood urea nitrogen; INR, International normalized ratio; PT, prothrombin time; PTT, partial thromboplastin time; RRT, renal replacement therapy; MAP, mean arterial pressure; RR, respiratory rate; LOS, length of stay; ICU, intensive care unit.

hazard ratio (HR) for the high BCR group was 1.7777 (95% CI: 1.4016–2.2547).

## 3.5 Subgroup analysis

Table 5 shows the results of the subgroup analysis. High BCR was consistently associated with the risk of in-hospital mortality among most subgroups (all $P$ for interaction >0.05), except for age (interaction $P = 0.042$), diabetes (interaction $P = 0.004$), and severe liver disease (interaction $P = 0.002$).

## 3.6 Analysis based on types of VTE (DVT and PE)

All VTE patients were divided into groups based on their diagnosis of either DVT or PE. The results were then analyzed

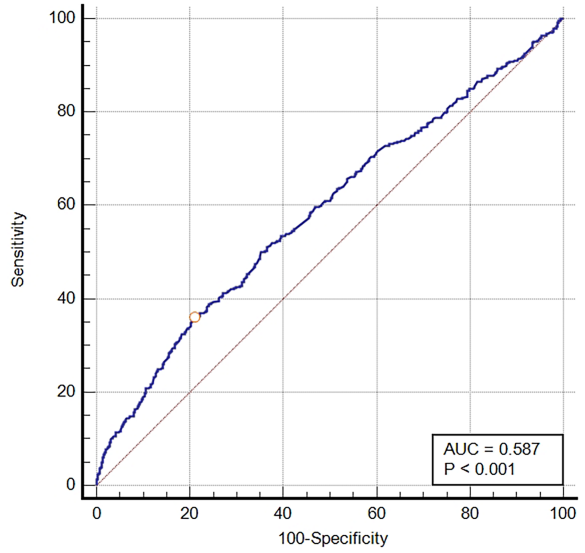


FIGURE 2
The ROC curve of predictive performance of BCR for in-hospital mortality. AUC, area under the curve; BCR, blood urea nitrogen to creatinine ratio; ROC, receiver operator characteristic curve.

separately for each group. Table 6 summarizes the baseline demographic and clinical characteristics of patients with DVT and PE. For patients with DVT, the comparison between survival and deceased cohorts revealed significant differences. Deceased patients were significantly older with higher CCI and SAPS II scores ($p < 0.001$). Laboratory tests showed decreased hemoglobin, RBC, and platelet levels but increased WBC, glucose, RDW, BUN, creatinine, and BUN/Cr ratio in deceased individuals (all $p < 0.05$). Moreover, deceased individuals demonstrated lower bicarbonate levels and higher INR PT and APTT values (all $p < 0.05$). Comorbidities such as congestive heart failure, malignant cancer, severe liver disease, and renal disease were more prevalent among deceased DVT patients ($p < 0.05$). Deceased DVT patients more often received renal replacement therapy ($p < 0.001$). Furthermore, deceased DVT patients had longer ICU stays ($p < 0.01$). DVT patients in the high BCR group (BCR > 26.84) demonstrated a significantly higher mortality rate compared to those in the low BCR group ($p < 0.001$) (Table 6). In patients with DVT, the crude logistic regression analysis showed that those in the high BCR group have a significantly higher risk of in-hospital mortality compared to those in the low BCR group, with an OR of 2.170 (95% CI: 1.612–2.921, $P < 0.001$). Even after adjusting for potential confounders, the association remained significant, with an adjusted odds ratio (aOR) of 1.872 (95% CI: 1.330–2.634; $p < 0.001$) (Supplementary Table S2). In patients with DVT, the Kaplan-Meier survival analysis revealed that the median survival time for the high BCR group was 44.840 days (95% CI: 35.315–80.825), which is significantly shorter than the 86.976 days (95% CI: 68.013–377.026) for the low BCR group. The high BCR group had an 81% higher risk of in-hospital mortality (HR:

TABLE 2 Baseline characteristics of BUN/creatinine ratio groups in patients with venous thromboembolism.

| Characteristics | Total ($n = 2,560$ | Low BCR ≤ 26.84 ($n = 1,966$) | High BCR >26.84 ($n = 594$) | *P* |
|---|---|---|---|---|
| Age, year | 64.5 (53–75.5) | 63 (51–74) | 69 (58–78) | <0.001 |
| Gender, male | 1,420 (55.5%) | 1,440 (55.5) | 221 (51.2) | 0.095 |
| CCI | 6 (4–8) | 5 (3–8) | 6 (5–8) | <0.001 |
| SAPS II Score | 36 (27–46) | 33 (25–44) | 40 (33–50) | <0.001 |
| Laboratory tests | | | | |
| Hemoglobin, g/dl | 10.7 (9.2–12.4) | 10.8 (9.4–12.6) | 10.1 (8.8–11.7) | <0.001 |
| WBC, 109/L | 11.2 (8.3–15.1) | 11.1 (8.3–14.8) | 11.6 (8.2–16.3) | 0.106 |
| RBC, 109/L | 3.6 (3.1–4.2) | 3.7 (3.2–4.2) | 3.4 (3.0–3.9) | <0.001 |
| Platelets, 109/l | 192 (133–264) | 194.4 (139.0–263.3) | 182.3 (114.2–265.8) | 0.001 |
| Glucose, mg/dl | 129 (109–160.5) | 129.0 (109.0–160.0) | 129.0 (110.0–165.0) | 0.331 |
| MCHC, g/dl | 33.1 (32.1–34.1) | 33.2 (32.2–34.2) | 32.9 (31.8–33.9) | <0.001 |
| RDW, % | 14.8 (13.7–16.5) | 14.6 (13.5–16.2) | 15.6 (14.3–17.6) | <0.001 |
| Hematocrit, % | 32.2 (28–37) | 32.7 (28.3–37.4) | 30.6 (27.1–35.7) | <0.001 |
| Bicarbonate, mEq/L | 23 (20–25.5) | 23.0 (20.0–25.0) | 23.0 (20.0–26.0) | 0.056 |
| Coagulation tests | | | | |
| INR | 1.3 (1.2–1.5) | 1.3 (1.2–1.5) | 1.4 (1.2–1.7) | <0.001 |
| PT | 14.4 (12.9–16.8) | 14.3 (12.8–16.5) | 14.9 (13.2–17.8) | <0.001 |
| PTT | 34.4 (28.8–59) | 37 (28.9–60.2) | 34.5 (28.2–55.9) | 0.026 |
| Comorbidities | | | | |
| Hypertension | 1,014 (39.6) | 778 (39.6) | 236 (39.7) | 0.945 |
| Diabetes | 623 (24.3) | 478 (24.3) | 145 (24.4) | 0.961 |
| Congestive heart failure | 593 (23.2) | 422 (21.5) | 171 (28.8) | 0.000 |
| Coronary artery disease | 464 (18.1) | 336 (17.1) | 128 (21.5) | 0.013 |
| Renal disease | 385 (15) | 317 (16.1) | 68 (11.4) | 0.005 |
| Severe liver disease | 246 (9.6) | 164 (8.3) | 82 (13.8) | <0.001 |
| Obesity | 334 (13) | 268 (13.6) | 66 (11.1) | 0.110 |
| Malignant cancer | 586 (22.9) | 417 (21.2) | 169 (28.5) | <0.001 |
| Cerebrovascular disease | 392 (15.3) | 314 (16) | 78 (13.1) | 0.092 |
| COPD | 649 (25.4) | 476 (24.2) | 173 (29.1) | 0.016 |
| Monitoring parameters | | | | |
| Heart rate, Bpm | 89 (78–102) | 89 (77–101) | 91 (78–103) | 0.084 |
| MAP, mmHg | 78 (71–86) | 79 (72–87) | 76 (69–82) | <0.001 |
| RR, breaths/minutes | 19 (17–23) | 19 (17–22) | 20 (17–23) | 0.015 |
| SpO2,% | 97 (95–98) | 97 (96–98) | 97 (95–98) | 0.159 |
| Intervention | | | | |
| Mechanical ventilation | 991 (38.5) | 757 (38.5) | 234 (39.4) | 0.697 |
| Diuretic use | 382 (14.9) | 273 (13.9) | 109 (18.4) | 0.007 |
| RRT | 205 (8) | 175 (8.9) | 30 (5.1) | 0.002 |
| Outcomes | | | | |
| Length of hospital stay, days | 12.2 (6.9–20.9) | 12.4 (7–20.9) | 11.8 (5.3–20.5) | 0.018 |
| Length of ICU stay, days | 3.2 (1.9–6.9) | 3 (1.9–6.3) | 4.7 (2.4–10.1) | <0.001 |
| In-hospital mortality, *n* (%) | 373 (14.6) | 239 (12.2) | 134 (22.6) | <0.001 |

Values are expressed as the median (Interquartile range) or *n* (%). CCI, Charlson Comorbidity Index; SAPS, the simplified acute physiology score; WBC, white blood cells; RBC, red blood cell; MCHC, mean corpuscular hemoglobin concentration; RDW, red blood cell distribution width; BUN, blood urea nitrogen; INR, international normalized ratio; PT, prothrombin time; PTT, partial thromboplastin time; RRT, renal replacement therapy; MAP, mean arterial pressure; RR, respiratory rate; LOS, length of stay; ICU, intensive care unit.

TABLE 3 Univariate logistic regression analyses for in-hospital mortality in patients with VTE.

| Variable | OR (95% CI) | *P* value |
|---|---|---|
| Age, year | 1.025 (1.018–1.033) | <0.001 |
| Gender, male | 0.850 (0.682–1.059) | 0.146 |
| CCI | 1.229 (1.187–1.272) | <0.001 |
| SAPS II Score | 1.064 (1.056–1.072) | <0.001 |
| Hemoglobin, g/dl | 0.849 (0.804–0.897) | <0.001 |
| WBC, 109/L | 1.014 (1.005–1.022) | 0.001 |
| RBC, 109/L | 0.629 (0.537–0.737) | <0.001 |
| Platelets, 109/L | 0.999 (0.998–1.000) | 0.014 |
| Glucose, mg/dl | 1.004 (1.003–1.006) | <0.001 |
| MCHC, g/dl | 0.822 (0.768–0.880) | <0.001 |
| RDW, % | 1.195 (1.149–1.242) | <0.001 |
| Hematocrit, % | 0.958 (0.941–0.976) | <0.001 |
| Bicarbonate, mEq/L | 0.899 (0.875–0.924) | 0.056 |
| INR | 1.495 (1.312–1.702) | <0.001 |
| PT | 1.041 (1.028–1.054) | <0.001 |
| PTT | 1.007 (1.003–1.011) | <0.001 |
| Hypertension | 0.822 (0.654–1.032) | 0.092 |
| Diabetes | 1.344 (1.053–1.715) | 0.018 |
| Congestive heart failure | 1.499 (1.174–1.913) | 0.001 |
| Coronary artery disease | 1.307 (0.998–1.711) | 0.052 |
| Renal disease | 1.718 (1.306–2.261) | <0.001 |
| Severe liver disease | 2.170 (1.589–2.964) | <0.001 |
| Obesity | 0.654 (0.452–0.945) | 0.024 |
| Malignant cancer | 2.379 (1.883–3.006) | <0.001 |
| Cerebrovascular disease | 1.254 (0.939–1.676) | 0.125 |
| COPD | 1.183 (0.926–1.513) | 0.179 |
| Heart rate, Bpm | 1.012 (1.005–1.019) | <0.001 |
| MAP, mmHg | 0.959 (0.948–0.970) | <0.001 |
| RR, breaths/minutes | 1.060 (1.034–1.087) | <0.001 |
| SpO2,% | 0.914 (0.876–0.954) | <0.001 |
| Mechanical ventilation | 1.729 (1.387–2.157) | <0.001 |
| Diuretic use | 1.605 (1.215–2.120) | 0.001 |
| RRT | 3.884 (2.850–5.295) | <0.001 |
| Length of hospital stay, days | 1.004 (0.998–1.009) | 0.216 |
| Length of ICU stay, days | 1.029 (1.018–1.040) | <0.001 |

VTE; venous thromboembolism; OR, odds ration; CCI, Charlson Comorbidity Index; SAPS, the simplified acute physiology score; WBC, white blood cells; RBC, red blood cell; MCHC, mean corpuscular hemoglobin concentration; RDW, red blood cell distribution width; BUN, blood urea nitrogen; INR, international normalized ratio; PT, prothrombin time; PTT, partial thromboplastin time; RRT, renal replacement therapy; MAP, mean arterial pressure; RR, respiratory rate; LOS, length of stay; ICU, intensive care unit.

1.8145, 95% CI: 1.3432–2.4510). The log-rank test indicated a statistically significant difference in survival between the two groups (Logrank $p < 0.0001$) (Supplementary Figure S1).

The comparison between survival and deceased cohorts revealed significant differences for patients with PE. Deceased individuals were older with higher CCI and SAPS II scores ($p < 0.001$). Laboratory findings indicated lower hemoglobin, RBC, and platelet levels but higher WBC, glucose, RDW, BUN, creatinine, and BUN/Cr ratio in deceased patients (all $p < 0.05$). Furthermore, deceased patients exhibited lower bicarbonate levels and higher INR and PT values (all $p < 0.05$). Comorbidities such as diabetes, congestive heart failure, coronary artery disease, malignant cancer, renal disease, and cerebrovascular disease were more prevalent among deceased PE patients (all $p < 0.05$). Increased usage of mechanical ventilation, diuretics, and renal replacement therapy was observed in deceased PE patients (all $p < 0.05$). Furthermore, deceased PE patients experienced shorter hospital and ICU stays (all $p < 0.05$). PE patients with a BCR greater than 26.84 had a significantly higher mortality rate compared to those with a lower BCR ($p < 0.001$) (Table 6). In patients with PE, the crude logistic regression analysis indicated that those in the high BCR group had a significantly higher risk of in-hospital mortality compared to those in the low BCR group, with an odds ratio (OR = 2.003, 95% CI: 1.363–2.943, $p < 0.001$). The significant association persisted even after adjusting for potential confounders, with an aOR of 1.585 (95% CI: 1.024–2.45, $p = 0.039$) (Supplementary Table S2). In patients with PE, the Kaplan-Meier survival analysis showed that the median survival time for the high BCR group was 61.306 days (95% CI: 40.380–61.306), significantly shorter than the low BCR group's 97.894 days (95% CI: 85.482–97.894). The high BCR group had an 83% higher risk of in-hospital mortality (HR: 1.8343, 95% CI: 1.2450–2.7028). The log-rank test confirmed a statistically significant difference in survival between the groups (Logrank $p = 0.0022$) (Supplementary Figure S2).

## 4 Discussion

To our knowledge, this study is the first to explore the relationship between BCR and in-hospital mortality among critically ill patients with VTE. Our findings indicate that higher BCR was associated with increased in-hospital mortality among critically ill patients, even after adjusting for potential confounding variables. Furthermore, the baseline BCR levels of the individuals in the deceased cohort were significantly greater than those in the surviving group. Notably, among patients with

TABLE 4 Multivariable logistic regression analyses for in-hospital mortality in patients with venous thromboembolism.

| Characteristic | Crude model | | Model I | | Model II | | Model III | |
|---|---|---|---|---|---|---|---|---|
| | OR (95% CI) | *p*-value | OR (95% CI) | *p*-value | OR (95% CI) | *p*-value | OR (95% CI) | *p*-value |
| BCR | 1.029 (1.020–1.038) | <0.001 | 1.024 (1.014–1.033) | <0.001 | 1.032 (1.023–1.042) | <0.001 | 1.016 (1.006–1.026) | 0.002 |
| Low BCR | 1 (Reference) | | 1 (Reference) | | 1 (Reference) | | 1 (Reference) | |
| High BCR | 2.105 (1.664–2.663) | <0.001 | 1.874 (1.475–2.381) | <0.001 | 2.091 (1.626–2.688) | <0.001 | 1.420 (1.086–1.857) | 0.010 |

The crude model did not adjust any of the variables.
Model I made adjustments to the variables of age and gender.
Model II made adjustments to 11 variables, including hypertension, diabetes, congestive heart failure, coronary artery disease, renal disease, severe liver disease, obesity, malignant cancer, mechanical ventilation, diuretic use, and renal replacement therapy.
Model III made further adjustments to 18 variables, including CCI, SAPS II Score, hemoglobin, WBC, RBC, platelets, glucose, MCHC, RDW, hematocrit, bicarbonate, INR, PT, PTT, HR, MAP, RR, and SpO2.

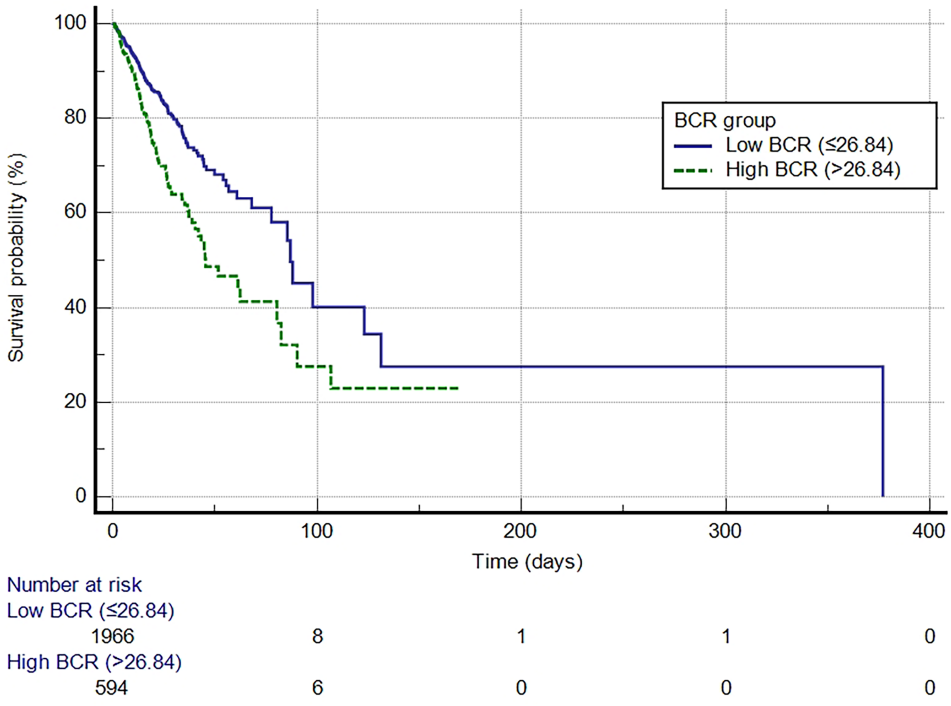


FIGURE 3
Kaplan-Meier survival curve for in-hospital mortality for the high and low BCR groups. BCR, blood urea nitrogen to creatinine ratio.

VTE in critical condition, a BCR greater than 26.84 was found to be an independent risk factor for in-hospital death. Compared to the low BCR group, patients in the high BCR group had significantly shorter median survival times, indicating that BCR may be a prognostic factor for VTE patients, with high BCR indicating a poor prognosis. Moreover, the hazard ratio for the high BCR group was 1.7777, indicating a 77.77% higher risk of in-hospital death in comparison to the low BCR group. Analysis based on VTE types also revealed that patients with high BCR had a significantly increased risk of in-hospital mortality in both the DVT and PE groups. This association remained statistically significant even after adjusting for numerous potential confounders.

Although the kidneys filter both BUN and creatinine, only BUN is reabsorbed in both the proximal and distal renal tubules. The activation of the neurohormonal system, which comprises the sympathetic nervous system, the renin-angiotensin-aldosterone system (RAAS), and vasopressin, impacts the reabsorption of BUN (12). Neurohormonal activation, triggered by conditions like heart failure, VTE, liver cirrhosis, or dehydration, can lead to an elevated BCR even without significant renal dysfunction (30). The assessment of BUN and creatinine levels is a routine practice in the ICU. The BCR is a straightforward and readily obtainable measure, as it relies solely on venous blood samples in clinical settings. Previous studies have reported an association between the BCR and in-hospital mortality in various diseases (17–20). BCR, a biomarker of neurohormonal activity, has been associated with poor prognosis in patients with acute heart failure and is an independent predictor of all-cause mortality (31). Impaired blood flow and endothelial dysfunction in heart failure contribute to a prothrombotic state, increasing the risk of thromboembolic events like stroke, intracardiac thrombi, VTE, PE, and myocardial infarction (32). Chen et al. (33) examined the link between BCR levels and in-hospital mortality among patients with subarachnoid hemorrhage, utilizing the MIMIC-IV database. Their study revealed a significant association, indicating that elevated BCR levels (≥27.208) were correlated with an increased risk of in-hospital mortality in comparison to lower BCR levels (<27.208). Another study by Han et al. (17) examined the correlation between BCR and all-cause mortality in adult patients with septic shock. They demonstrated that even after adjusting for potential confounders, a high BCR (≥27.3 mg/dl) in patients with septic shock was significantly linked to all-cause mortality. Ok et al. (34) investigated the utility of the BUN/Cr ratio upon admission in predicting disease severity and survival rates for patients with COVID-19. Their investigation revealed that a higher BUN/Cr ratio served as an independent prognostic indicator for both the COVID-19 severity and patient outcomes related to survival. Ma et al. (20) conducted a recent investigation to elucidate the association between the BUN/Cr ratio and in-hospital mortality among patients diagnosed with trauma-induced acute respiratory distress syndrome. Their findings revealed a statistically significant correlation between elevated BUN/Cr ratios and an increased risk of death within the hospital setting for these patients. Additionally, the study

TABLE 5 Subgroup analysis of the association between BCR and in-hospital mortality in VTE patients.

| Subgroup | *N* | OR (95% CI) High BCR >26.84 | *P* for interaction |
|---|---|---|---|
| Gender | | | 0.282 |
| Male | 1,420 | 2.371 (1.703–3.301) | |
| Female | 1,140 | 1.830 (1.309–2.559) | |
| Age | | | 0.042 |
| <65 | 1,280 | 2.722 (1.853–3.998) | |
| ≥65 | 1,280 | 1.639 (1.215–2.210) | |
| Hypertension | | | 0.359 |
| Yes | 1,014 | 2.432 (1.656–3.571) | |
| No | 1,546 | 1.937 (1.438–2.609) | |
| Diabetes | | | 0.004 |
| Yes | 623 | 1.173 (0.728–1.889) | |
| No | 1,937 | 2.601 (1.979–3.419) | |
| Congestive heart failure | | | 0.096 |
| Yes | 593 | 1.510 (0.976–2.335) | |
| No | 1,967 | 2.340 (1.769–3.095) | |
| Coronary artery disease | | | 0.084 |
| Yes | 464 | 3.067 (1.868–5.038) | |
| No | 2,096 | 1.863 (1.422–2.440) | |
| Renal disease | | | 0.251 |
| Yes | 385 | 1.595 (0.877–2.900) | |
| No | 2,175 | 2.327 (1.796–3.015) | |
| Severe liver disease | | | 0.002 |
| Yes | 246 | 0.849 (0.457–1.577) | |
| No | 2,314 | 2.374 (1.839–3.063) | |
| Obesity | | | 0.363 |
| Yes | 334 | 1.470 (0.653–3.307) | |
| No | 2,226 | 2.162 (1.690–2.768) | |
| Malignant cancer | | | 0.650 |
| Yes | 586 | 1.857 (1.245–2.770) | |
| No | 1,974 | 2.083 (1.550–2.801) | |
| Cerebrovascular disease | | | 0.180 |
| Yes | 392 | 3.055 (1.717–5.434) | |
| No | 1,862 | 1.978 (1.527–2.561) | |
| COPD | | | 0.066 |
| Yes | 649 | 1.471 (0.939–2.306) | |
| No | 1,911 | 2.403 (1.823–3.168) | |

The reference group was Low BCR group; BCR, Blood urea nitrogen to creatinine ratio; VTE, venous thromboembolism.

demonstrated a superior predictive value of the BUN/Cr ratio in predicting in-hospital mortality compared to using BUN or creatinine levels alone. The aforementioned results suggest that a higher BCR is linked to more severe conditions compared to a lower BCR. Our results concur with the above findings. In this cohort study utilizing the MIMIC-IV database, we found that the optimal cut-off value of BCR to predict survival status among patients with VTE was 26.84. Moreover, our findings demonstrated that the BCR serves as an independent predictor of in-hospital death in critically ill VTE patients. Additionally, our results showed a strong association between low BCR levels and improved survival outcomes. Patients in the low BCR group exhibited a statistically significant longer median survival time (86.976 days) than those in the high BCR group (45.477 days). Notably, compared to those with lower BCR levels, a higher BCR level (>26.84) upon admission was correlated with an increased risk of in-hospital mortality in critically ill VTE patients. While additional research is required for a comprehensive understanding of the underlying mechanisms, the elevated BCR in VTE patients could potentially be associated with several mechanisms. Firstly, VTE has been associated with prolonged hypoxemia and hypercapnia, potentially leading to activation of the RAAS (35, 36). While the mechanism is not fully elucidated, this activation may contribute to increased BUN levels through tubular reabsorption of urea.. Secondly, patients with VTE often have concomitant cardiovascular comorbidities, including heart failure (37, 38). In the context of cardiovascular disease, a multifaceted neurohormonal response triggers activation of the renal sympathetic nervous system and the RAAS, potentially leading to altered urea reabsorption (12). Thirdly, VTE is associated with persistent neutrophilia and the subsequent release of proinflammatory mediators due to hypoxia (26). These mediators may disrupt cardiorenal function and neurohumoral regulation, potentially leading to increased BUN levels (30). Finally, VTE can indirectly lead to elevated BUN and creatinine levels by causing blood stasis and reducing blood flow to the kidneys (30).

In the present study, the overall in-hospital mortality of the study population was 14.6%. A study by Ambra et al. (39) in Qatar reported a 13.39% mortality rate for hospitalized patients with venous VTE. Another retrospective cohort study conducted at seven major hospitals in Saudi Arabia demonstrated that the mortality rate for patients with confirmed VTE was 14.3% (40). Using an extensive ICU database, Pisani et al. (41) assessed the risk of VTE in critically ill pneumonia patients and revealed that patients with VTE had a high mortality rate (20.6%). This wide variability in mortality rates among patients with VTE can be attributed to several factors, including the patient population, severity of VTE, comorbidities, and timeliness of diagnosis and treatment. Our findings emphasize the importance of carefully monitoring BCR in VTE patients admitted to ICUs. Since BCR is a simple parameter obtainable from routine clinical blood tests, it may enable clinicians to promptly identify VTE patients prone to adverse outcomes, necessitating early intensive care intervention. There are several notable strengths in this study. To our knowledge, it is the first to investigate the potential association between BCR and in-hospital mortality among VTE patients using a large dataset from a heterogeneous population in the ICU. Furthermore, even after adjusting for various confounding factors in three different models, the relationship between the BCR and mortality remained consistent, suggesting the robustness and stability of our results. The BCR is a readily available index for risk assessment in critically ill patients, as BUN and creatinine are part of routine blood tests. Furthermore, this research suggests clinicians can enhance prognostic accuracy for patients with VTE by utilizing this affordable and easily accessible biomarker.

This study has several limitations. Firstly, although multivariable analyses were employed, the findings of this study could still be affected by residual bias and unmeasured confounding factors due to its retrospective nature. Secondly, retrospective studies have inherent limitations, such as potential

TABLE 6 Demographic and clinical characteristics of patients with deep vein thrombosis and pulmonary embolism.

| Characteristics | Deep vein thrombosis | | | Pulmonary embolism | | |
|---|---|---|---|---|---|---|
| | Survival ($n = 1,366$) | Death ($n = 231$) | *P* | Survival ($n = 821$) | Death ($n = 142$) | *P value* |
| Age, year | 63 (53–74) | 70 (60–80) | <0.001 | 64 (51–75) | 69 (60–78) | <0.001 |
| Gender, male | 822 (60.2%) | 132 (57.1%) | 0.385 | 404 (49.2) | 62 (43.7) | 0.222 |
| CCI | 5 (4–7) | 8 (6–10) | <0.001 | 5 (3–7) | 7 (5–10) | <0.001 |
| SAPS II Score | 35 (26–43) | 48 (38–61) | <0.001 | 32 (24–42) | 50 (38–61) | <0.001 |
| Laboratory tests | | | | | | |
| Hemoglobin, g/dl | 10.7 (9.4–12.5) | 10.1 (8.8–11.3) | <0.001 | 10.9 (9.3–12.5) | 9.8 (8.5–11.9) | 0.001 |
| WBC, $10^9$/L | 11 (7.9–14.6) | 12.2 (8.2–18.3) | 0.007 | 11.2 (8.6–14.5) | 13.9 (9.5–18.9) | <0.001 |
| RBC, 109/L | 3.6 (3.1–4.1) | 3.3 (2.9–3.8) | <0.001 | 3.7 (3.2–4.3) | 3.4 (2.9–4.1) | <0.001 |
| Platelets, 109/L | 185.1 (129.5–252) | 161 (93.3–248) | 0.001 | 211.2 (152.5–279) | 192.8 (110–273.7) | 0.022 |
| Glucose, mg/dl | 128.3 (108.5–159.5) | 137.5 (111.5–167.3) | 0.041 | 126.5 (108.5–158) | 137.8 (113–192) | <0.001 |
| MCHC, g/dl | 33.4 (32.4–34.4) | 32.9 (31.8–34) | <0.001 | 32.7 (31.8–33.7) | 32.3 (31.4–33.3) | 0.001 |
| RDW, % | 14.6 (13.6–16.2) | 16.5 (14.8–18.4) | <0.001 | 14.6 (13.5–16.2) | 15.6 (14.4–17.4) | <0.001 |
| Hematocrit, % | 32.1 (28.1–36.9) | 30.3 (26.5–34.6) | <0.001 | 33.2 (28.7–37.8) | 31.1 (26.9–36.6) | 0.007 |
| BUN, mg/dl | 18.5 (13–30) | 30 (18.5–46.5) | <0.001 | 17 (12.5–25.5) | 24 (15–37) | <0.001 |
| Creatinine, ng/dl | 1 (0.8–1.4) | 1.3 (0.9–2.2) | <0.001 | 0.9 (0.7–1.2) | 1.1 (0.8–1.7) | <0.001 |
| BUN/Cr ratio | 18.5 (14–25.4) | 22.2 (14.9–31.9) | <0.001 | 19 (14.3–25) | 21.1 (15.6–29.8) | 0.002 |
| Bicarbonate, mEq/L | 23 (20.5–25.5) | 21.5 (18–24) | <0.001 | 23 (20.5–25.5) | 21.5 (18–24.5) | <0.001 |
| Coagulation tests | | | | | | |
| INR | 1.3 (1.2–1.5) | 1.5 (1.3–2) | <0.001 | 1.3 (1.2–1.5) | 1.4 (1.2–1.7) | <0.001 |
| PT | 14.4 (12.8–16.6) | 16.4 (14–21.7) | <0.001 | 14.1 (12.8–16) | 15.1 (13.3–17.8) | <0.001 |
| PTT | 33.9 (28.2–49.2) | 41.1 (31.6–59.1) | <0.001 | 42.4 (29.1–76.6) | 41.8 (29.7–87.8) | 0.395 |
| Comorbidities | | | | | | |
| Hypertension | 563 (41.2%) | 74 (32%) | 0.008 | 318 (38.7) | 59 (41.5) | 0.526 |
| Diabetes | 341 (25.0) | 68 (29.4) | 0.150 | 173 (21.1) | 41 (28.9) | 0.039 |
| Congestive heart failure | 285 (20.9) | 62 (26.8) | 0.042 | 197 (24.0) | 49 (34.5) | 0.008 |
| Coronary artery disease | 278 (20.4) | 48 (20.8) | 0.881 | 105 (12.8) | 33 (23.2) | 0.001 |
| Renal disease | 207 (15.2) | 55 (23.8) | 0.001 | 97 (11.8) | 26 (18.3) | 0.032 |
| Severe liver disease | 168 (12.3) | 57 (24.7) | <0.001 | 16 (1.9) | 5 (3.5) | 0.236 |
| Obesity | 150 (11) | 16 (6.9) | 0.062 | 149 (18.1) | 19 (13.4) | 0.167 |
| Malignant cancer | 264 (19.3) | 90 (39) | <0.001 | 181 (22.0)) | 51 (35.9 | <0.001 |
| Cerebrovascular disease | 222 (16.3) | 35 (15.2) | 0.674 | 103 (12.5) | 32 (22.5) | 0.002 |
| COPD | 318 (23.3) | 60 (26.0) | 0.373 | 226 (27.5) | 45 (31.7) | 0.308 |
| Monitoring Parameters | | | | | | |
| Heart rate, Bpm | 87 (76–100) | 92 (82–104) | <0.001 | 92 (79–103) | 95 (80–106) | 0.163 |
| MAP, mmHg | 78 (71–86) | 73 (68–81) | <0.001 | 80 (73–89) | 75 (69–83) | <0.001 |
| RR, breaths/minutes | 19 (17–22) | 21 (17–24) | <0.001 | 20 (18–23) | 22 (19–25) | 0.003 |
| SpO2,% | 97 (96–99) | 97 (95–99) | 0.010 | 97 (95–98) | 96 (95–98) | 0.266 |
| Intervention | | | | | | |
| Mechanical ventilation | 554 (40.6) | 109 (47.2) | 0.059 | 250 (30.5) | 78 (54.9) | <0.001 |
| Diuretic use | 187 (13.7) | 42 (18.2) | 0.072 | 118 (14.4) | 35 (24.6) | 0.002 |
| RRT | 99 (7.2) | 52 (22.5) | <0.001 | 32 (3.9) | 22 (15.5) | <0.001 |
| Outcomes | | | | | | |
| LOS hospital, day | 12.7 (7.3–21.2) | 13.5 (6.7–23.5) | 0.687 | 11.9 (6.8–20.5) | 8.3 (3.7–17) | <0.001 |
| LOS ICU, day | 3.1 (1.9–6.4) | 5 (2.5–10.6) | <0.001 | 3 (1.9–6.2) | 4.2 (2.3–8.9) | 0.001 |
| BCR category | | | | | | |
| High BCR > 26.84 | 289 (21.2) | 85 (36.8) | <0.001 | 171 (20.8) | 49 (34.5) | <0.001 |

OR, odds ratio; CCI, Charlson Comorbidity Index; SAPS, the simplified acute physiology score; WBC, white blood cells; RBC, red blood cell; MCHC, mean corpuscular hemoglobin concentration; RDW, red blood cell distribution width; BUN, blood urea nitrogen; INR, international normalized ratio; PT, prothrombin time; PTT, partial thromboplastin time; RRT, renal replacement therapy; MAP, mean arterial pressure; RR, respiratory rate; LOS, length of stay; ICU, intensive care unit.

missing data and an inability to establish causality. These factors may impact the validity and interpretation of our findings. Thirdly, owing to constraints within the MIMIC-IV database, information regarding several factors affecting the BCR, such as the administration of corticosteroids and specific antibiotics, protein consumption, and muscle mass, were not evaluated in this study because they could not be extracted from the MIMIC-IV database. Fourthly, the ROC curve analysis was used to identify the optimal threshold value for the BCR, with the maximal Youden index serving as the predictor of survival status. Nevertheless, the area under the curve for BCR was smaller than expected. Finally, the MIMIC-IV database lacks detailed information regarding mortality causes and specific treatments such as anticoagulation and thrombolysis, which restricts our

ability to assess potential differences in therapeutic approaches among VTE patients. Therefore, prospective cohort studies are warranted to validate these findings. Finally, this was a single-center study based on data obtained from the MIMIC-IV database, limiting our findings' generalizability.

## 5 Conclusions

Our findings demonstrate that the BCR serves as an independent predictor of mortality within the hospital setting for critically ill patients diagnosed with VTE. Patients presenting with elevated BCR levels exhibited a significantly increased risk of in-hospital mortality compared to those with lower BCR levels. BCR is a highly practical, readily available, and relatively inexpensive index that could be a valuable tool for clinicians in managing and stratifying the risk for critically ill VTE patients at an early stage.

## Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: The Medical Information Mart for Intensive Care IV (MIMIC-IV) database at https://physionet.org/content/mimiciv/2.2/.

## Ethics statement

The studies involving humans were approved by Institutional Review Boards (IRB) of the Massachusetts Institute of Technology (MIT, Cambridge, MA, USA) and Beth Israel Deaconess Medical Center. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

## Author contributions

AP: Writing – review & editing, Writing – original draft, Visualization, Software, Resources, Methodology, Investigation, Funding acquisition, Formal Analysis, Data curation, Conceptualization. MG: Writing – review & editing, Writing – original draft, Validation, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal Analysis, Data curation, Conceptualization. HH: Writing – review & editing, Writing – original draft, Visualization, Software, Project administration, Methodology, Investigation, Formal Analysis, Conceptualization. QZ: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal Analysis, Conceptualization.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2024.1400915/full#supplementary-material

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# Advancements in the interventional therapy and nursing care on deep vein thrombosis in the lower extremities

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This review examines recent advancements in interventional treatments and nursing care for lower extremity deep vein thrombosis (DVT), highlighting significant innovations and their clinical applications. It discusses the transition to novel anticoagulants such as Direct Oral Anticoagulants, which offer a safer profile and simplified management compared to traditional therapies. Mechanical interventions, including balloon angioplasty and venous stenting, are detailed for their roles in improving immediate and long-term vascular function in acute DVT cases. Furthermore, the use of image-guided techniques is presented as essential for enhancing the accuracy and safety of DVT interventions. Additionally, this study outlines advances in nursing care strategies, emphasizing comprehensive preoperative and postoperative evaluations to optimize patient outcomes. These evaluations facilitate tailored treatment plans, crucial for managing the complex needs of DVT patients. Long-term care strategies are also discussed, with a focus on patient education to ensure adherence to treatment protocols and to prevent recurrence. The synthesis aims to inform healthcare professionals about cutting-edge practices in DVT management, promoting a deeper understanding of how these advancements can be integrated into clinical practice. It also underscores the necessity for ongoing research to address challenges such as cost-effectiveness and patient compliance, ensuring that future treatments are both accessible and effective.

KEYWORDS

**deep vein thrombosis, lower extremity, interventional therapy, anticoagulants, nursing care**

## 1 Introduction

Deep vein thrombosis (DVT) represents a significant medical condition characterized by the formation of blood clots within the major veins of the lower extremities (1, 2). This condition not only induces pain, swelling, and limited mobility in the affected limb but also poses a severe risk of life-threatening pulmonary embolisms should a clot dislodge and

migrate to the lungs (1, 2). Given its prevalence and the severe complications associated with it, DVT is a critical public health issue demanding effective strategies for its prevention, diagnosis, and management (3).

Traditionally, the management of DVT has centered around anticoagulant therapy, the use of compression stockings, and occasionally, surgical interventions (4). While these treatments have proven beneficial, they come with notable limitations (5). Anticoagulants, for example, while effective in preventing clot progression and new clot formation, require rigorous monitoring to mitigate the risk of severe bleeding complications. Compression stockings are beneficial for reducing thrombus formation and edema but often face challenges with patient compliance (6). Furthermore, these conventional approaches do not fully address the recurrence of the condition or the long-term complications associated with DVT (6).

In response to these challenges, there has been a marked shift towards more innovative interventional treatments and care protocols in recent years (7). Advanced treatment modalities, including novel anticoagulants, mechanical thrombectomy, and image-guided minimally invasive procedures, have been developed (8, 9). These innovations offer enhanced efficacy and safety, showing particular promise in the management of severe or complex DVT cases.

The objective of this mini review is to conduct a thorough evaluation and synthesis of the current scientific and clinical advancements in the interventional treatment and management of DVT. This mini review will scrutinize the latest research findings and clinical trials, aiming to elucidate how these innovative approaches have refined patient outcomes. Moreover, it explores prospective research avenues and potential advancements in technology that could further revolutionize the treatment landscape of DVT.

This review is crucial for healthcare professionals as it not only highlights contemporary treatment methodologies but also lays down a comprehensive framework for future investigations. Such rigorous examination is essential for advancing clinical practice and enhancing patient care in the realm of DVT.

# 2 Recent advances in interventional therapy of DVT in lower extremities

## 2.1 Pharmacological interventions

Pharmacological interventions play a pivotal role in the management of DVT, with significant advancements in both drug types and therapeutic strategies in recent years. Modern pharmacotherapy primarily involves anticoagulants and thrombolytics, which have substantially evolved to improve both efficacy and safety for patients (10–18) (Table 1).

### 2.1.1 Anticoagulation therapy

The advent of direct oral anticoagulants (DOACs), including rivaroxaban, apixaban, and dabigatran, marks a significant shift from traditional warfarin therapy (10–18) (Table 1). DOACs offer advantages such as reduced need for monitoring, fewer dietary and drug interactions, and a more stable therapeutic profile. Not requiring frequent INR monitoring, these anticoagulants simplify outpatient management and enhance patient adherence.

Clinical trials such as EINSTEIN, RE-COVER, and AMPLIFY have demonstrated the efficacy of DOACs in treating DVT with a notably lower risk of bleeding compared to traditional anticoagulants. These studies underline the role of DOACs in setting new standards for anticoagulation therapy (10–18).

Alongside established therapies, the development of anti-XI anticoagulants marks an innovative frontier in thromboprophylaxis (28). These agents specifically inhibit Factor XI, offering a targeted approach to reducing thrombosis risk while potentially minimizing bleeding complications—a common limitation of traditional anticoagulants (28, 29). Emerging clinical trials have begun to explore the efficacy and safety profiles of these anticoagulants, indicating their promise for safer therapeutic regimens in populations at high risk of bleeding (30). The introduction of anti-XI agents underscores the dynamic evolution of anticoagulant therapy, aiming to balance efficacy and safety more effectively (31).

### 2.1.2 Thrombolytic therapy

Tissue plasminogen activator, such as alteplase, continues to be extensively utilized for acute DVT management, effectively dissolving clots and restoring venous flow (14, 15) (Table 1). Its application is crucial in managing severe thrombotic events where rapid resolution is necessary.

The ATTRACT trial, among others, has explored the role of thrombolytics in the early intervention of DVT to prevent post-thrombotic syndrome and chronic venous insufficiency. While highlighting the benefits in symptom reduction, these studies also stress the importance of careful patient selection to mitigate bleeding risks.

## 2.2 Mechanical interventional treatments

The development of mechanical interventional techniques has introduced novel therapeutic possibilities for DVT, especially beneficial for patients who are not ideal candidates for conventional anticoagulation (19, 20) (Table 1).

### 2.2.1 Balloon angioplasty

This technique involves the insertion of a catheter with an attached balloon into the thrombosed vein (21). Upon inflation, the balloon physically expands the vein, displacing the clot and restoring blood flow. This approach is particularly effective in acute cases where rapid amelioration of symptoms is critical (21) (Table 1). Balloon angioplasty is typically reserved for critical cases requiring rapid restoration of venous flow due to significant obstruction (21). This intervention is particularly crucial in severe phlegmasia dolens, where there is an immediate risk of limb ischemia.

### 2.2.2 Venous stenting

Stenting involves the placement of a metallic mesh within the vein to maintain vascular patency and prevent future occlusions. It is particularly applicable in scenarios where venous stenosis persists post-thrombosis or when angioplasty alone does not suffice to restore normal blood flow (19, 22). The stent acts as a scaffold, ensuring

TABLE 1 Interventional therapy in patients with DVT in the lower extremities.

| Reference | Type of study | Treatment | Key findings |
|---|---|---|---|
| Schulman et al. (10) | Randomized Controlled Trial | Dabigatran vs. warfarin | A fixed dose of dabigatran is as effective and safe as warfarin and does not require laboratory monitoring. |
| Agnelli et al. (11) | Randomized Controlled Trial | Apixaban | A fixed-dose regimen of apixaban was noninferior to conventional therapy for acute VTE and resulted in significantly less bleeding. |
| Prins et al. (12) | Observational Study | Rivaroxaban | Rivaroxaban showed similar efficacy to standard therapy with a significantly lower rate of major bleeding. |
| Trca et al. (13) | Randomized Controlled Trial | Pentasaccharide medication | Potential protective effects of pentasaccharides against DVT development may be expected. |
| Okuda et al. (14) | Randomized Controlled Trial | Heparin and intermittent pneumatic compression | Prevention of DVT post-laparoscopic cholecystectomy. |
| Verhaeghe et al. (15) | Pilot Study | Systemic rt-PA | Efficacy and safety in treating DVT of lower extremities. |
| Fronas et al. (16) | Observational Study | Rivaroxaban | Feasibility of rivaroxaban in deferred workup of suspected DVT. |
| Napolitano et al. (17) | Randomized Controlled Trial | Low molecular weight heparin | Six months of LMWH lowers thrombosis recurrence risk; extending treatment in those with residual vein thrombosis does not further reduce VTE. |
| Chang et al. (18) | Observational Study | Alteplase | Intraclot injection of alteplase directly into the thrombus is an alternative to continuous infusion, reducing systemic exposure to thrombolytics. |
| Meng et al. (19) | Randomized Control Trial | Stenting post-thrombolysis | Stenting iliac vein obstructions after CDT for lower extremity DVT may improve deep vein patency, enhancing efficacy and quality of life. |
| Snyder et al. (20) | Randomized Control Trial | Mechanical compression device and aspirin | Prevention efficacy post-total knee arthroplasty. |
| Li et al. (21) | Retrospective Study | Balloon-assisted CDT | Balloon-assisted CDT is a superior, safer treatment for acute DVT, reducing medication, procedure time, and increasing patency without raising PE risk. |
| Sukovatykh et al. (22) | Observational Study | Venous stenting | Stenting in acute period of total and subtotal DVT. |
| Comerota et al. (23) | Retrospective Study | CDT | Improved health-related quality of life in iliofemoral DVT. |
| Davidson et al. (24) | Observational Study | Color Doppler sonography | Screening for DVT in asymptomatic postoperative patients. |
| Vedantham et al. (25) | Consensus Report | Endovascular treatment | Reporting standards for endovascular treatment of DVT. |
| Kim et al. (26) | Observational Study | CT imaging | DVT density's clinical value for predicting pulmonary thromboembolism. |
| Mani et al. (27) | Randomized Control Trial | MRV and gadofosveset | MRV with gadofosveset trisodium reliably estimates thrombus volume in DVT in a multicenter setting. |

DVT, deep vein thrombosis; VTE, venous thromboembolism; LMWH, low molecular weight heparin; CDT, catheter-directed thrombolysis; MRV, magnetic resonance venography.

long-term patency and improving overall venous return, which is crucial for reducing recurrence rates and improving long-term outcomes (19, 22). Stenting is primarily recommended for managing chronic venous insufficiency, particularly when compressive symptoms continue to persist despite the application of less invasive treatments (32).

Recent clinical data affirm that mechanical interventions like balloon angioplasty and stenting significantly enhance symptom management and quality of life (19, 22) (Table 1). However, the sustainability of treatment effects and the potential for long-term complications necessitate further research and careful patient follow-up.

## 2.3 Image-guided interventional strategies

Image-guided techniques have revolutionized the precision and safety of interventions for managing DVT, proving essential in both diagnosis and therapeutic application.

### 2.3.1 Ultrasound guidance

Color Doppler ultrasound is the frontline modality for diagnosing DVT, providing real-time, dynamic blood flow information. It is indispensable for guiding minimally invasive procedures such as catheter-directed thrombolysis, allowing for

direct visualization of the thrombus and treatment effects (23–25) (Table 1).

Advancements in Ultrasound Technology: The field of ultrasound technology has seen significant advancements, notably in the development of portable, high-resolution devices (33). These improvements have expanded the reach of ultrasound beyond traditional clinical settings, facilitating its use in remote locations and potentially increasing patient access to timely diagnosis and management.

Future Directions in Ultrasound and Digital Health Integration: Looking forward, the integration of ultrasound technology with digital health applications holds promising potential for enhancing patient management (34). Innovations on the horizon include wearable devices capable of continuous monitoring of vital parameters such as heart rate, oxygen saturation, and electrocardiographic signals (34). These devices could play a crucial role in early detection of complications, such as pulmonary embolism, and enhance preventive care. Furthermore, advancements are expected in the development of smart medication adherence tools that remind patients of dosage schedules, thereby improving compliance with treatment protocols (35).

These technological advancements are poised to transform the management of DVT, enhancing both the efficacy and safety of treatments and supporting proactive patient care through continuous monitoring and timely intervention.

### 2.3.2 Advanced imaging techniques

Computed tomography (CT) or magnetic resonance imaging (MRI) offers comprehensive imaging capabilities that extend beyond the limitations of ultrasound, especially useful in complex cases where clots are located in anatomically challenging positions (26, 27). These modalities enable detailed visualization of the vascular anatomy, facilitating precise interventions such as stenting and targeted thrombolysis, ensuring interventions are performed with the highest accuracy (26, 27) (Table 1).

Specifically, the use of CT and MRI is highly restricted and generally recommended in specialized cases such as left iliac DVT in pregnant patients (26, 27). In these patients, MRI is preferred over CT due to the absence of ionizing radiation, which poses a risk to the developing fetus (27). This ensures that both mother and fetus are safeguarded while obtaining essential diagnostic information to guide further treatment.

The integration of image-guided technology not only enhances the effectiveness of therapeutic interventions but also minimizes procedural risks, thereby improving patient safety and treatment outcomes. As imaging technology advances, future interventions are expected to become even more refined, further elevating the standard of care for patients with DVT.

## 3 Recent advances in nursing strategies of DVT in lower extremities

### 3.1 Preoperative and postoperative care

Effective preoperative and postoperative care is crucial in optimizing outcomes and minimizing complications in the interventional treatment of lower extremity DVT. Advancements in medical technology have greatly enhanced the precision of nursing assessments, which now play a pivotal role in the planning and execution of treatment (Table 2).

Nursing Assessments: Modern nursing practices involve comprehensive evaluations using standardized tools that assess hemorheology, limb measurements, and a patient's overall physiological and psychological state. These evaluations help clinicians identify patient-specific risk factors and predict potential complications, allowing for the creation of tailored interventional treatment plans (6, 36). Furthermore, assessments extend to evaluating a patient's understanding and preparedness for upcoming treatments, ensuring they are fully informed and ready both mentally and physically.

Preoperative Preparations: Key aspects of preoperative care include thorough health examinations to ascertain the patient's baseline health status and detailed discussions to gauge their understanding and acceptance of the proposed interventions (37–39). Educating patients about what to expect during and after the procedure is critical. This education covers potential postoperative scenarios and adherence to prescribed self-care protocols.

Postoperative Management: Focuses on managing pain and overseeing wound care, essential for facilitating a swift recovery and optimizing the treatment's success. Pain management strategies often encompass both pharmacological and non-pharmacological approaches, including physical therapy (40). Wound care involves regular monitoring and timely intervention if signs of infection arise, preventing further complications. Postoperative care also includes functional rehabilitation and ongoing psychological support, fostering a holistic recovery approach.

Implementing these refined care strategies can significantly enhance patient outcomes, reducing the risks and complications associated with the treatment of DVT (Table 2).

### 3.2 Long-term care and patient education

Managing lower extremity DVT extends beyond immediate medical intervention and encompasses long-term care and patient education, which are essential for the sustained well-being of patients (Table 2).

Home Care: After hospital discharge, home care becomes a critical aspect of a DVT patient's routine, particularly during the recovery and maintenance phases. Patients need comprehensive instructions and continuous support for activities such as adhering to anticoagulation regimens and monitoring for adverse effects (41, 42). The ongoing anticoagulation is crucial for preventing thrombus reformation but carries an inherent risk of bleeding, necessitating patient education on managing these risks effectively (41, 42).

Lifestyle Modifications: Adjustments in daily living, such as maintaining an active lifestyle, achieving and sustaining an appropriate weight, and following a heart-healthy diet, are crucial (43). These modifications help mitigate the risk of recurrent DVT and enhance overall health.

TABLE 2 Nursing strategies in patients with DVT in the lower extremities.

| Reference | Type of study | Treatment | Key findings |
|---|---|---|---|
| Kearon et al. (6) | Practice Guidelines | Antithrombotic therapy for VTE | Provided guidelines for antithrombotic therapy in VTE. |
| Heit et al. (36) | Retrospective Study | Not applicable | This underscores the need to identify at-risk patients and understand VTE to ensure effective prophylaxis. |
| CLOTS Trials Collaboration (37) | Randomized Controlled Trial | Thigh-length graduated compression stockings | Evaluated effectiveness in preventing DVT post-stroke. |
| Ho et al. (38) | Meta-analysis | Intermittent pneumatic compression | Prevented VTE in hospitalized patients. |
| Kakkos et al. (39) | Systematic Review | Combined intermittent pneumatic compression and pharmacological prophylaxis | Prevention of VTE. |
| Rawal (40) | Review Article | Not applicable | Discussed current issues in postoperative pain management. |
| Kahn et al. (41) | Observational Study | Not applicable | Analyzed determinants and time course of postthrombotic syndrome. |
| Wells et al. (42) | Diagnostic Study | Evaluation of D-dimer | Assessed the utility of D-dimer in diagnosing suspected DVT. |
| Pezold et al. (43) | Case Report | Percutaneous mechanical thrombectomy | Described thrombectomy in a pediatric patient with DVT. |
| Crişan et al. (44) | Descriptive Study | Online methods of patient education | Evaluated effectiveness of online education for DVT patients. |

DVT, deep vein thrombosis; VTE, venous thromboembolism.

Patient Education: This is an integral component of long-term management, empowering patients with knowledge about their condition, the importance of their treatment regimen, and the ability to recognize symptoms of potential complications (44). Education should not only provide disease and treatment-related information but also include practical advice on when and how to seek further medical assistance (44). Effective education transforms patients from passive recipients of healthcare into active participants in their health management, aiming to prevent recurrence and elevate their quality of life.

By enhancing these aspects of care, healthcare providers can ensure better management of DVT, promoting patient independence and improving outcomes in long-term disease management.

## 4 Nursing strategies for special populations in the treatment of DVT in lower extremities

Elderly Patients: Elderly individuals undergoing DVT intervention often present with multiple comorbidities, such as cardiovascular diseases and renal insufficiency, which can affect their response to anticoagulant therapy (45). The diminished drug metabolism typical in older age further elevates the risk of adverse reactions during treatment. Hence, nursing care for elderly patients should include meticulous monitoring of drug dosages and therapeutic responses. Care plans must also consider potential cognitive impairments and the availability of family support, tailoring education and support programs to meet their specific needs. This ensures that elderly patients receive care that is both effective and sensitive to their particular health status.

Pregnant Women: DVT treatment in pregnant women requires careful consideration of both maternal and fetal health due to the teratogenic risks associated with many standard anticoagulants (46–48). The selection and timing of therapeutic interventions must prioritize safety, avoiding any potential harm to the developing fetus. Physiological changes during pregnancy can alter the risk profile for thrombus formation and modify responses to treatment, necessitating vigilant monitoring and management (46–48). Collaborative efforts with obstetric specialists are essential to tailor interventions that safeguard the health of both mother and child, ensuring that treatment protocols are precisely followed.

Patients with Chronic Diseases: Individuals with chronic conditions such as cancer, diabetes, or cardiovascular disease may face additional challenges when undergoing DVT interventions (3, 49). These conditions can influence both the choice of treatment and the overall prognosis. For instance, cancer patients are particularly susceptible to increased thrombotic risk due to the malignancy and associated treatments like chemotherapy. Nursing care strategies must be adaptive to these risks, adjusting therapeutic approaches to effectively manage and minimize the likelihood of recurrence. It is also crucial for the care team to address the comprehensive needs of these patients, including nutritional support, effective pain management, and psychological care, to enhance their overall quality of life and treatment outcomes.

In summary, effective nursing care for special populations undergoing treatment for lower extremity DVT requires a detailed

understanding of each individual's unique medical and personal circumstances. By implementing personalized and meticulously planned care strategies, healthcare providers can ensure that interventions are not only safe and effective but also aligned with the specific needs and conditions of elderly patients, pregnant women, and those with chronic diseases. This approach promotes optimal health outcomes and patient satisfaction.

# 5 Challenges and future directions in the interventional treatment of DVT in lower extremities

Despite notable advancements in the management of lower extremity DVT through interventional treatments, several challenges persist that affect the adoption and efficacy of these approaches.

## 5.1 Technological limitations

Current interventional techniques such as thrombectomy and catheter-directed thrombolysis are effective but involve complex procedures that require a high degree of technical skill (3, 50, 51). The reliance on sophisticated medical equipment also limits the application of these treatments in resource-constrained settings (3, 50, 51). Addressing these technological constraints involves simplifying procedures and developing equipment that is both cost-effective and suitable for use in a broader range of healthcare environments.

## 5.2 Cost-effectiveness

The high costs associated with interventional treatments for DVT pose significant barriers to their widespread use (41, 49–53). These costs can be prohibitive for patients and healthcare systems, especially in lower-income areas. Future research must focus on conducting thorough cost-effectiveness analyses to identify economic strategies that can reduce expenses while maintaining or enhancing clinical outcomes. Exploring generic medication options, streamlined procedural techniques, and cost-sharing models could be viable approaches.

## 5.3 Patient acceptance

The acceptance of new or complex interventional treatments by patients is crucial for successful outcomes (41, 54). Challenges in patient education and the communication of treatment benefits and risks are significant (41, 54). Improving educational strategies to better inform patients about their options, the procedures involved, potential risks, and expected outcomes is essential. Enhanced communication efforts should aim to build trust and understanding, facilitating informed consent and engagement in treatment decisions.

## 5.4 Future research and innovations

Research in the coming years may concentrate on creating simpler, more affordable interventional techniques. There is a need to innovate in patient education and care coordination to adapt to the evolving landscape of healthcare delivery. Future developments could include (55–57):

Technology Advancements: Redesigning devices and simplifying interventional procedures to make them more accessible and easier to perform.

Digital Tools: Leveraging technology to enhance patient education, treatment transparency, and engagement through digital platforms that provide accessible information and support.

Interdisciplinary Approaches: Developing collaborative care models that integrate various healthcare professionals, including doctors, nurses, physical therapists, and social workers, to deliver holistic and coordinated care.

Such innovations are expected to optimize the management of DVT, significantly improve clinical outcomes, and broaden the application of interventional treatments, making them more accessible and effective across diverse patient populations and geographic locations.

# 6 Summary

A thorough evaluation of the recent developments in interventional treatments and nursing strategies for lower extremity DVT reveals several critical insights. Firstly, technological advancements in interventional methods have notably increased the efficiency and safety of treatments for DVT. The adoption of advanced anticoagulants, mechanical thrombectomy techniques, and image-guided minimally invasive surgeries has shown exceptional effectiveness in clinical settings, particularly in managing complex or high-risk DVT cases. Furthermore, enhancements in nursing strategies, including detailed assessments before and after surgery, effective pain management, and comprehensive long-term care coupled with patient education, have substantially improved both the rehabilitation outcomes and the overall quality of life for patients.

Despite these advancements, the need for continuous research is imperative. Future investigations should delve deeper into the capabilities of emerging technologies, especially focusing on their role in minimizing complications and fostering long-term wellness for patients. Moreover, there is a critical need to focus on increasing patient compliance with treatment regimens and improving the effectiveness of interdisciplinary collaborations to streamline and execute treatment protocols more efficiently. By addressing these areas, we can further refine DVT treatment processes and enhance the quality of care provided to patients, ultimately leading to significant improvements in their quality of life.

# Author contributions

C-yJ: Conceptualization, Data curation, Methodology, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. D-dD: Data curation, Methodology, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. X-yB:

Conceptualization, Methodology, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. XZ: Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. Y-nW: Conceptualization, Data curation, Investigation, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



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# The radiological diagnosis of pregnancy associated venous thromboembolism: a review of current research

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One of the main causes of unfavorable pregnancy outcomes in expectant mothers is pregnancy-associated venous thromboembolism. Although pregnancy-related venous thromboembolism does not always manifest obvious clinical symptoms and lacks a comprehensive standard risk assessment and prediction system as well as simple and effective laboratory testing techniques, timely and accurate diagnosis can still help reduce the probability of adverse pregnancy outcomes. To aid in the early detection, diagnosis, and treatment of pregnancy- associated venous thromboembolism, we attempt to provide an overview of the radiological diagnostic techniques for various forms of the condition.

KEYWORDS

**venous thromboembolism, pregnancy, deep vein thrombosis, pulmonary embolism, radiological diagnosis**

## 1 Introduction

It is commonly known that one of the main factors contributing to pregnant women's higher complications and fatality rates is pregnancy-associated venous thromboembolism (PA-VTE). The disease's occurrence has increased due to the specificity of the physiological status of women who are pregnant or just gave birth (Venous stagnation, hypercoagulable state of blood, and pelvic vascular endothelial injury caused by childbirth, etc.), as well as the absence of a thorough, standardized method for assessing PA-VTE risk (1). Existing PA-VTE risk assessment models typically focus only on VTE risk factors validated by large-scale studies (2, 3), yet using these factors to assess PA-VTE risk often proves inadequate. The physiological and pathological conditions of pregnant and postpartum women differ from those of non-pregnant individuals, and their physical and mental states undergo dynamic changes throughout pregnancy. Existing PA-VTE risk assessment models often employ static risk factors, such as age, gender, body mass index (BMI), past medical history, genetic history, lifestyle and so on. Thus, while data show that the application of these models has reduced maternal deaths to some extent, their predictive accuracy and precision still require enhancement. Some high-risk pregnant women may be underestimating their risk of developing PA-VTE, potentially leading to irreversible severe outcomes, while some low-risk women may be overestimating their risk, resulting in excessive antithrombotic therapy. According to a study conducted in more than 100 national hospitals, the rate of maternal death attributable to PA-VTE was 3.2% (4). The percentage of elderly pregnant women and *in vitro* fertilization (IVF) has increased dramatically due to the growing demands placed on today's

populations as well as scientific and technological advancements. The incidence and negative outcomes of PA-VTE in assisted reproductive technology pregnancies have grown in comparison to natural pregnancies, regardless of the technology's success (5). According to studies by Zhao et al. (6), the majority of pregnant women who encounter PA-VTE do not receive early screening and prevention in prenatal care for a variety of reasons. There are still signs to look out for and treatment options that may be taken to lessen the likelihood of an unfavorable prognosis, even if the incidence rate of PA-VTE is substantial and causes more unfavorable obstetric outcomes. PA-VTE has also drawn more interest in recent years. Studies that are pertinent to the topic have demonstrated that prompt diagnosis and sensible intervention techniques can aid in the treatment of PA-VTE and even stop it from developing, which lowers the risk of unfavorable obstetric events. An overview of the development of PA-VTE diagnostic research is given in this article.

## 2 The concept of venous thromboembolism

The improper coagulation of blood in a vein that leads to the creation of a venous thrombus is known as venous thromboembolism (VTE). The detached thrombus, also known as a thrombus, travels into other organs with the flow of venous blood. This obstructs the blood arteries in the corresponding organ and prevents normal blood circulation, resulting in the occurrence of thrombotic diseases which may have no exhibit overt clinical symptoms. Pulmonary embolism (PE) and deep vein thrombosis (DVT) are the two main categories under which VTE falls (7). DVT is the term for abnormal blood coagulation in deep veins that causes embolism by obstructing the venous return channel at the coagulation site. Although research has indicated that DVT frequently happens in the deep veins of the lower limbs, it can also occasionally occur in other venous systems, Harnik et al. documented cases of mesenteric venous thrombosis, for example (8). PE is the term used when liquid, solid, or gas components obstruct one or more pulmonary arteries. PE is primarily brought on by blood clots that originate in the deep veins of the legs or pelvis, break off, and travel to the lungs via the venous return (9, 10). Studies have demonstrated that women who ultimately undergo cesarean section due to factors such as unsuitability for natural childbirth or personal choice are more likely to develop PE, and that PE is more likely to directly result in maternal mortality when compared to DVT (11).

## 3 The etiology of venous thromboembolism

Currently, endothelial damage, blood in a hypercoagulable state, and venous blood stasis are the three recognized components of thrombosis, sometimes known as the Virchow triad. Pregnant women's blood physiological changes perfectly match the aforementioned risk factors (12). According to available data, pregnant women's levels of coagulation factors significantly alter during pregnancy. In contrast to non-pregnant situations, the concentrations of coagulation factors V, VII, VIII, IX, X, XII, and vascular hemophilia factor increased, and there was a considerable decrease in the activity of protein S, which has physiological anticoagulant effects. The body's fibrinolytic activity is also momentarily impacted during pregnancy, but these hematological indicators swiftly restore to their pre-pregnancy levels following delivery (13). Pregnant women who experience any of these circumstances are likely to be hypercoagulable during pregnancy. According to research by James et al. (14), pregnant women can be somewhat protected by this hypercoagulable state, which can also lessen or prevent heavy bleeding during childbirth or miscarriage. However, pregnant women are also more likely to develop VTE than non-pregnant women, with the risk being more than four times higher (15). Multiple pregnancies, advanced age, cesarean sections, and other variables have also been linked to an increased chance of developing PA-VTE, according to a significant number of previous research (16). Taller pregnant women are more likely than the general population to have venous thrombosis during pregnancy because of studies that have demonstrated that they have more venous valves as a result of their bigger venous surface area (17, 18).

## 4 Diagnosis of venous thromboembolism

According to Zhang et al.'s research, the riskiest time to develop PA-VTE is one week following delivery (19). The diagnosis of PA-VTE is made more challenging by the findings of Elgendy et al.'s study, which showed that many clinical symptoms and indicators of the condition are comparable to physiological changes that occur during pregnancy, such as lower limb edema, dyspnea, and tachycardia (20). As a result, in clinical practice, diagnosing PA-VTE requires combining patient complaints with imaging tests. However, because pregnant and postpartum women are unique, there are additional factors that need to be taken into account when performing imaging examinations. These include whether the examination will have an impact on fetal growth and development, its detection rate, and how convenient it will be for both the examination physicians and the pregnant and postpartum patients.

### 4.1 Diagnosis of DVT

The left lower limb accounts for the great majority of DVT cases during pregnancy, which may be connected to the anatomical and physiological location of the left common iliac vein. It is situated in the space between the fifth lumbar vertebra and the right common iliac artery. It is narrower than the right common iliac vein, has a sluggish blood flow velocity, and is prone to vortex formation, all of which can increase the risk of venous thrombosis. Color Doppler ultrasonography (CUS) testing should be done initially when clinical physicians suspect DVT in pregnant women presenting with clinical symptoms of inguinal pain, unilateral leg pain, or edema (21). Women who are pregnant or recently gave birth typically accept CUS more readily because it is a quick, affordable, environmentally friendly, radiation-free, repeatable test that can be completed in a short amount of time. CUS examination is considered a traditional diagnostic approach for DVT, with a study indicating that the diagnosis rate of DVT through CUS is roughly 98.7% (22). Despite the fact that multi-slice CT venography (MSCTV) and contrast-enhanced venography (CV) offer incredibly high diagnosis rates and a sharper depiction of venous orientation. However, these tests are typically invasive and need for the use of radioactively infused contrast chemicals. According to certain criteria, CUS tests, which have a high sensitivity and

specificity, can generally substitute CV and MSCTV examinations in the diagnosis of DVT (23). A CUS examination is sufficient for the majority of pregnant women suspected of having DVT; nevertheless, some pregnant women show normal results on the CUS examination but continue to have comparable clinical symptoms. In order to rule out or diagnose DVT in these groups, a UK study recommends that either a magnetic resonance imaging (MRI) or a CUS test be done for a week (21). Additionally, MRI examination does not necessitate the use of radioactive contrast agents or exposure to ionizing radiation during the examination process. In comparison to CUS examination, it can achieve high-quality imaging with computer-aided assistance and better visualize the vena cava and pelvic veins (24). The study by Torkzad et al. (25) also suggests that MRI examination typically has a higher detection rate in diagnosing DVT of the pelvic vein, and even within the population enrolled in this research, a patient with a thrombus that had migrated to the inferior vena cava only received positive results through MRI examination. A case report by Dronkers et al. (26) on iliac vein thrombosis further supports the importance of MRI in diagnosing DVT of the pelvic vein.

## 4.2 Diagnosis of PE

The well-known trinity of pulmonary embolism (PE) symptoms—chest discomfort, hemoptysis, and dyspnea—are frequently utilized as early clinical indicators to diagnose PE. However, this is frequently hampered by typical physiological reactions that occur during pregnancy, such as physiological dyspnea and chest discomfort (20, 27). According to a study conducted in the US, PE affects about 370,000 people yearly and may eventually result in 60,000–100,000 fatalities (10). Clinical physicians should exercise caution when dealing with the aforementioned symptoms and have the necessary examinations as soon as feasible to rule out PE because of the high mortality risk of PE (28). Evidence-based diagnostic recommendations for PE have been developed by the American Thoracic Society and the American College of Obstetricians and Gynecologists (29). According to the guidelines, patients should initially get a chest X-ray examination if they have matching concerns. It is commonly recognized that chest X-ray exams are inexpensive and simple to perform, making them a popular choice for radiation exams in clinical settings. The positive diagnosis rate of a chest X-ray was only 59% in a research with 436 confirmed PE patients (30), and the limited specificity of this diagnostic technique means that it is not as frequently employed in clinical practice for PE diagnosis or differentiation. This examination technique is frequently used by clinicians as a PE screening tool. It is advised to have a ventilation perfusion (V/Q) examination if a chest X-ray is negative for pregnant and postpartum women who continue to report pertinent clinical symptoms in order to prevent missed diagnosis. While radioactive contrast chemicals, like iodohexanol, are necessary for pulmonary V/Q assessment, pregnant women and fetuses may be exposed to radioactive nuclides as a result of this procedure (31, 32). Nonetheless, research has demonstrated that the dose of radioactive isotopes utilized for lung V/Q evaluation is far less than the dose that may affect the fetus and mother (33). Therefore, we still advise that patients with clinical symptoms but a negative chest X-ray obtain further lung V/Q testing in order to diagnosis or rule out PE, taking into account the benefits and drawbacks of doing so. Clinicians also need to consider whether the examination may lead to over-testing for patients, resulting in over-treatment of small blood clots that do not yet require treatment, as these treatment procedures are bound to pose potential risks to pregnant women and foetuses. Simultaneously, research has indicated that lung perfusion scanning can be carried out prior to lung V/Q assessment. In addition to lowering the amount of radioactive isotopes that the mother and fetus are exposed to as a result of this examination, performing lung ventilation scanning again is not necessary if the examination results are normal (34). Clinical physicians must do Computed Tomography Pulmonary Angiography (CTPA) on patients exhibiting clinical symptoms if their chest X-ray scan yields good results. Previous research has demonstrated that CTPA is a highly sensitive and negative predictive value test for PE diagnosis. As a result, CTPA is frequently utilized as the "gold standard" for PE diagnosis in real-world clinical settings (35). However, some research also suggests that lung V/Q test, which is appropriate for ruling out PE, has the same effect as CTPA (36). The choice between X-ray examination, V/Q scan, or CTPA in clinical practice may depend more on the experience of physicians and the individual preferences of patients and their families because there is a dearth of evidence on whether examinations offer clear advantages.

# 5 Future outlook

The aforementioned information indicates that PA-VTE, which has a variety of etiological variables and physiological traits specific to pregnant women, is a key contributor to the rise in the global rate of maternal death. Clinical practitioners have many constraints when choosing critical imaging examination techniques during diagnosis because of the physiological characteristics of maternal women and fetal. Due to physiological changes, such as uterine enlargement and compression of blood vessels during pregnancy, pregnant women often experience symptoms like lower limb swelling and pain, which can interfere with the initial diagnosis of PA-VTE based on clinical symptoms. While existing diagnostic imaging techniques offer a reliable basis for the diagnosis of PA-VTE to a certain extent, they still have several limitations. For instance, CUS lacks sensitivity for detecting small blood clots in blood vessels, and CTPA is an invasive procedure that requires the use of radioactive isotopes, making it unsuitable and undesirable for all pregnant and postpartum women to undergo such examinations.

However, with the patient's informed agreement, we can still carefully carry out some important imaging examinations that will not significantly affect the mother and fetus after carefully weighing the benefits and disadvantages. This is made possible by the advancement of medical technology. The diagnosis of PA-VTE can also benefit from laboratory testing, such as measuring blood levels of D-dimer, which indicates coagulation status. Nonetheless, it's critical to establish particular diagnostic standards catered to the requirements of pregnant women, considering the physiological rise in D-dimer levels during pregnancy. In order to better protect pregnant women and fetuses, we aspire to find more environmentally friendly, secure, and highly accurate examination techniques in the future. In order to decrease false positives, clinical physicians can collaborate with laboratory specialists to improve the specificity of hematological monitoring markers. They can also work with radiologists to explore optical scanning diagnostic techniques, like Optical coherence

tomography (OCT), with the goal of improving the safest methods for detecting small and elusive venous thrombi. To create PA-VTE diagnostic criteria and risk assessment prediction models specifically for expectant mothers, obstetricians and gynecologists can work with the departments of hematology, laboratory, and radiology.

## Author contributions

DY: Writing – original draft. LW: Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



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# Risk factors for postoperative DVT exacerbation or new DVT in patients with spinal cord injury complicated with cervical fracture

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**Objective:** To investigate the dynamic changes of perioperative deep venous thrombosis (DVT) in patients with spinal cord injury complicated with cervical fracture and analyze the risk factors of postoperative DVT exacerbation or new DVT.
**Methods:** From January 2018 to December 2023, a total of 232 patients with spinal cord injury complicated with cervical fracture in our hospital were retrospectively analyzed. Ultrasonography of both lower limbs was performed before and after surgery. According to whether there was aggravation of DVT or new thrombosis, the group was divided into DVT exacerbation or new DVT group and non-DVT exacerbation group. Clinical data were used to study the dynamic changes of perioperative DVT. Logistic regression analysis and receiver operating characteristic (ROC) curve were used to explore the risk factors.
**Results:** The DVT was found in 47 patients before surgery, including 26 cases of distal thrombus, 14 cases of mixed thrombus and 7 cases of proximal thrombus. Postoperative DVT increased to 81, including 31 distal thrombus, 35 mixed thrombus and 15 proximal thrombus. A total of 53 patients (22.8%, 53/232) experienced postoperative DVT exacerbation or new DVT. Logistic regression analysis revealed that age, American Spinal Injury Association (ASIA) score, time from injury to surgery, surgery time and blood loss were risk factors for postoperative DVT exacerbation.
**Conclusions:** In patients with spinal cord injury complicated with cervical fracture, the risk of postoperative DVT exacerbation is very high. Age, ASIA score A-B, time from injury to surgery, surgery time and blood loss were risk factors for postoperative DVT exacerbation or new DVT.

KEYWORDS

deep venous thrombosis, spinal cord injury, cervical fracture, risk factor, thrombosis exacerbation

## Introduction

Cervical fracture with acute spinal cord injury (SCI) is common following cervical spine trauma and is associated with a number of complications (1). The prevalence of SCI with cervical fracture is 10%–51% (2–4). Cervical fracture with SCI can result in high mortality, severe disability, central nervous pain, and significant socioeconomic burden (5–7). Patients with acute SCI are at high risk for venous thromboembolism (VTE), which includes DVT and pulmonary embolism (PE). The incidence of VTE in patients with acute SCI is 12%–64%, and the mortality rate is 9.7% (8, 9).

The DVT refers to abnormal coagulation of blood in the deep venous cavity, blocking the venous lumen and causing venous obstruction, which is commonly seen in the lower limbs (10). Although DVT has obvious clinical manifestations such as pain in the early stage, the main complaints of patients with SCI are not obvious due to partial or complete loss of superficial sensation. 50%−80% of patients with DVT had no specific clinical symptoms in the early stage, and over 50% of patients had normal physical examinations, leading to a high rate of missed diagnosis (11). So, there are significant differences in the epidemiology of DVT in patients with SCI reported by different studies.

Many studies have investigated risk factors associated with DVT in patients with cervical SCI (12–16). Previous studies mainly focus on static DVT after SCI. But to our knowledge, after the formation of DVT in patients with SCI, only a few thrombi are confined to the site of occurrence or disappear spontaneously, and most of them extend to the deep venous trunk of the entire limb, which can cause various complications such as post-thrombotic syndrome, edema, compression ulcer and even PE (17–19). There are no reports on the dynamic change of DVT and the risk factors of thrombosis aggravation in patients with cervical SCI.

The purpose of this study was to investigate the dynamic change of perioperative DVT in patients with SCI complicated with cervical fracture and analyze the risk factors of postoperative DVT exacerbation or new DVT.

# Patients and methods

## Study population

This retrospective study analyzed the data of patients with acute SCI complicated with cervical fractures admitted to Baoding No.1 Central Hospital from January 2018 to December 2023. Finally, a total of 232 patients were enrolled, including 175 males and 57 females. The causes of injuries included 114 cases of high fall injuries, 82 cases of traffic accidents, 14 cases of fall at ground level and 22 cases of injury caused by heavy object. The study inclusion criteria were as follows: (1) acute SCI patients with cervical fractures admitted to our hospital within 1 week of injury caused by trauma; (2) complete medical records. The exclusion criteria were as follows: (1) chronic SCI patients(>1 week); (2) combined with multiple organ injury; (3) abnormality of the coagulation function; (4) anticoagulation before injury; (5) previous history of VTE; (6) accompanied by other sites of fractures. This study was approved by the Ethics Committee of our hospital and our study obtained the informed consent of all patients.

## Diagnosis, prevention and grouping of DVT

Ultrasonography was conducted to diagnose DVT. The examination encompassed all veins in the lower extremities, including the external iliac vein, femoral vein, popliteal vein, posterior tibial vein, fibular vein, and intermuscular vein. All examinations were performed by qualified radiologists using the same ultrasound equipment (Philips Medical Healthcare, Armstrong, Netherlands). All patients underwent lower limb venous ultrasonography before and 3–5 days after surgery. The diagnosis of DVT is based on the Guidelines for Diagnosis and Treatment of Deep Venous Thrombosis (Third Edition) proposed by the Chinese Medical Association (20). The DVT was categorized into three distinct types: central DVT (occurring in the popliteal vein or proximal regions), peripheral DVT (localized distally to the popliteal vein), and mixed DVT (involving both central and peripheral thrombosis).

All patients in this study received both mechanical and chemical prophylaxis after admission (21). Low molecular weight heparin (LMWH) (4,100 U, once per day, Changshan Production, Hebei, China) was subcutaneously injected to prevent DVT. Mechanical prophylaxis, such as intermittent pneumatic compression and ankle pump training, was also implemented. Patients with DVT received twice daily treatment with LMWH and immediately stopped mechanical prophylaxis. It was up to the vascular surgeon to decide whether to use an inferior vena cava filter. The anticoagulation strategy remained unchanged after surgery.

Patients were divided into DVT exacerbation or new DVT group and non-DVT exacerbation group based on dynamic change in thrombosis detected by ultrasonography. The DVT exacerbation or new DVT group was defined as a new proximal thrombus, a new mixed thrombus, a new distal thrombus, a single thrombus developing into a double thrombus, and a distal thrombus progressing to a proximal thrombus or mixed thrombus.

## Data collection

Demographic and clinical data were collected, including age, gender, height, weight, body mass index (BMI), comorbidities (including hypertension, diabetes and coronary heart disease), ASIA score, time from injury to surgery, causes (including traffic accident, high fall, injury caused by heavy object, and fall at ground level), surgery time, blood loss, liquid transfusion, blood transfusion, surgical procedures (anterior surgery or posterior surgery), D-dimer, fibrinogen(FIB), prothrombin time(PT), thrombin time(TT), activated partial thromboplastin time(APTT).

## Statistical analysis

Statistical analyses were conducted with SPSS (Version 22.0, IBM SPSS Inc., Chicago, IL, USA). Continuous variables with normal distribution were given as mean ± standard deviation (SD) and compared using independent *t* tests. Non-normally distributed continuous variables were represented as median values (interquartile range, IQR) and examined using the Mann-Whitney *U*-test. Categorical variables were presented as proportions and compared using the chi-squared test. The variables showing $p < 0.05$ were selected into the multivariate logistic analysis which explored risk factors for DVT exacerbation. ROC curve analyses were also used to evaluate the diagnostic value of risk factors. *P*-values <0.05 were considered statistically significant.

# Results

## Perioperative change of DVT before and after surgery

174 patients (75.00%, 174/232) had no change in thrombus before and after surgery (including 5 patients with proximal thrombus, 11 patients with mixed thrombus, 10 patients with distal thrombus and 148 patients without thrombus). Among 37 patients without thrombus before surgery (15.95%, 37/232), there were 6 cases of proximal thrombus, 16 cases of mixed thrombus, and 15 cases of distal thrombus after surgery. Four patients (1.72%, 4/232) with distal thrombus changed to proximal thrombus after surgery, and nine patients (3.88%, 9/232) changed to mixed thrombus. Three patients (1.29%, 3/232) of mixed thrombus became distal thrombus after surgery, and 1 patient had thrombus disappearance after surgery (Table 1).

## Univariate analysis of DVT exacerbation or new DVT

As shown in Table 2, the average age in the DVT exacerbation or new DVT group was 57.94 ± 11.38 whereas that in the non-DVT exacerbation group was 44.07 ± 13.10. The patients with DVT

TABLE 1 Dynamic changes of DVT before and after surgery.

| Preoperative thrombosis | Cases(*n*) | Postoperative thrombosis | | | |
|---|---|---|---|---|---|
| | | Proximal thrombus | Distal thrombus | Mixed thrombus | No thrombus |
| Proximal thrombus | 7 | 5 | 1 | 1 | 0 |
| Distal thrombus | 26 | 4 | 11 | 9 | 2 |
| Mixed thrombus | 14 | 0 | 3 | 10 | 1 |
| No thrombus | 185 | 6 | 16 | 15 | 148 |
| Total | 232 | 15 | 31 | 35 | 151 |

TABLE 2 Patient characteristics between the two groups.

| Variables | DVT exacerbation or new DVT Group (*n* = 53) | Non-DVT exacerbation Group (*n* = 179) | *P* Value |
|---|---|---|---|
| Age(years) | 57.94 ± 11.38 | 44.07 ± 13.10 | <0.001 |
| Gender(male/female) | 38/15 | 137/42 | 0.472 |
| BMI(kg/m$^2$) | 24.12 ± 1.96 | 23.80 ± 2.14 | 0.325 |
| Comorbidities | | | |
| Hypertension(%) | 12/53 (22.64%) | 54/179 (30.17%) | 0.286 |
| Diabetes(%) | 7/53 (13.21%) | 27/179 (15.08%) | 0.734 |
| Coronary heart disease(%) | 2/53 (3.77%) | 11/179 (6.15%) | 0.510 |
| Causes | | | |
| Traffic accident | 22 (41.51%) | 60 (33.52%) | 0.612 |
| Fall at ground level | 4 (7.55%) | 10 (5.58%) | |
| Injury caused by heavy object | 5 (9.43%) | 17 (9.50%) | |
| High fall | 22 (41.51%) | 92 (51.40%) | |
| ASIA score | | | |
| A–B | 25 (47.17%) | 24 (13.41%) | <0.001 |
| C–D | 28 (52.83%) | 155 (86.59) | |
| Surgery time(min) | 163.74 ± 47.23 | 124.97 ± 32.15 | <0.001 |
| Blood loss(ml) | 680 (400) | 424 (300) | <0.001 |
| Liquid transfusion(ml) | 2,269.81 ± 396.06 | 2,167.04 ± 510.76 | 0.179 |
| Blood transfusion(yes/no) | 23/30 | 50/129 | 0.033 |
| Surgical procedures | | | |
| Anterior surgery | 41 (77.36%) | 138 (77.09%) | 0.968 |
| Posterior surgery | 12 (22.64%) | 41 (22.91%) | |
| Time from injury to surgery(days) | 7.62 ± 2.93 | 5.51 ± 2.68 | <0.001 |
| D-dimer(mg/L) | 2.60 (2.01) | 1.88 (1.67) | 0.011 |
| FIB(g/L) | 3.58 (1.29) | 3.43 (1.23) | 0.357 |
| TT(s) | 14.31 (2.05) | 14.61 (2.20) | 0.662 |
| APTT(s) | 29.49 (5.35) | 29.17 (4.90) | 0.537 |
| PT(s) | 12.25 (1.50) | 11.93 (1.50) | 0.226 |

BMI, body mass index; DVT, deep vein thrombosis; ASIA, American Spinal Injury Association; FIB, fibrinogen; PT, prothrombin time; TT, thrombin time; APTT, activated partial thromboplastin time.

TABLE 3 Multivariable analysis of the risk factors for DVT exacerbation.

| Risk factors | β | SE | Wald | Exp (β) | 95% CI | *P* Value |
|---|---|---|---|---|---|---|
| Age(years) | 0.098 | 0.021 | 22.616 | 1.103 | 1.059–1.149 | <0.001 |
| ASIA score | 1.364 | 0.489 | 7.785 | 3.912 | 1.501–10.197 | 0.005 |
| Surgery time(min) | 0.020 | 0.006 | 12.550 | 1.020 | 1.009–1.031 | <0.001 |
| Time from injury to surgery(days) | 0.310 | 0.075 | 16.973 | 1.363 | 1.176–1.580 | <0.001 |
| Blood loss(ml) | 0.001 | 0.001 | 4.835 | 1.001 | 1.000–1.003 | 0.028 |

ASIA, American Spinal Injury Association.

exacerbation were older than those without DVT exacerbation ($P < 0.001$). There was a significant difference in ASIA score between the two groups ($P < 0.001$). Time from injury to surgery was significantly different between the two groups: $7.62 \pm 2.93$ vs. $5.51 \pm 2.68$, respectively ($P < 0.001$). there were also significant differences in surgery time, blood loss, blood transfusion, and D-dimer between the two groups (all $P < 0.05$). However,there were no significant differences between the two groups in gender, BMI, comorbidities, causes, surgical procedures, FIB, TT, APTT, and PT (all $P > 0.05$).

## Multivariate analysis of DVT exacerbation or new DVT

Multivariate logistic regression was performed to identify risk factors. As shown in Table 3, the results showed that age (OR, 1.103; 95%CI:1.059–1.149; $P < 0.001$), ASIA score A-B (OR, 3.912; 95%CI:1.501–10.197; $P = 0.005$), time from injury to surgery (OR, 1.363; 95% CI:1.176–1.580; $P < 0.001$), surgery time (OR, 1.020; 95% CI:1.009–1.031; $P < 0.001$), and blood loss (OR, 1.001; 95% CI:1.000–1.003; $P = 0.028$) were risk factors for postoperative DVT exacerbation.

## ROC analysis of different risk factors

The ROC curves for the five risk factors were shown in Figure 1. The value of AUC (area under the ROC curve) usually represents the diagnostic value of risk factors. The diagnostic value of age (AUC value = 0.788) was the highest one among these factors. The AUC values of ASIA score, time from injury to surgery, surgery time, and blood loss were 0.669, 0.720, 0.750 and 0.740, respectively (Table 4). The diagnostic cut-off values were determined by the Youden index and were more than 5

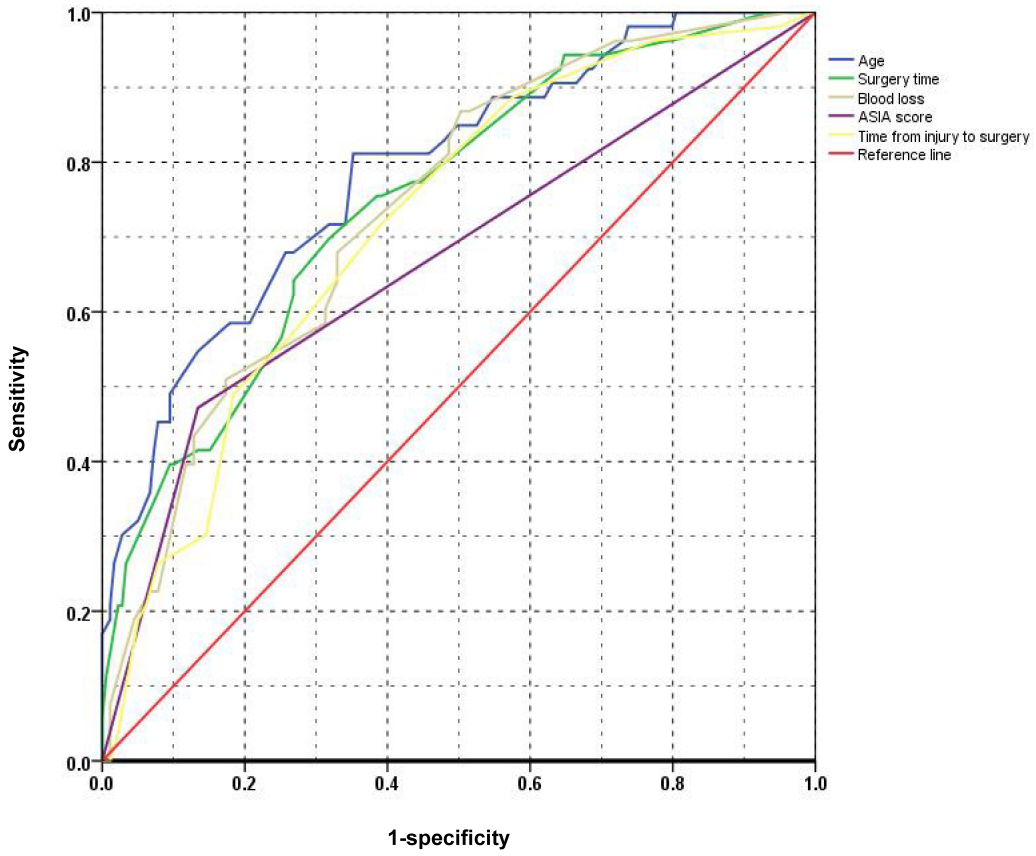


FIGURE 1
The ROC curves of different risk factors.

TABLE 4 The ROC results of different factors.

| Risk factors | Cut-off value[a] | Sensitivity | Specificity | AUC | 95% CI | SE | *P* Value |
|---|---|---|---|---|---|---|---|
| Age(years) | 50 | 81.13% | 64.80% | 0.788 | 0.730–0.839 | 0.036 | <0.001 |
| ASIA score | – | 47.17% | 86.59% | 0.669 | 0.604–0.729 | 0.037 | <0.001 |
| Surgery time(min) | 135 | 69.81% | 68.16% | 0.750 | 0.690–0.805 | 0.037 | <0.001 |
| Time from injury to surgery(days) | 5 | 71.70% | 60.89% | 0.720 | 0.657–0.777 | 0.038 | <0.001 |
| Blood loss(ml) | 330 | 86.79% | 49.72% | 0.740 | 0.678–0.795 | 0.037 | <0.001 |

ASIA, American Spinal Injury Association.
[a]The cut-off points of scores were determined by the Youden index.

days from injury to surgery, ASIA score A-B, 135 min of surgery time and 330 ml of blood loss, respectively.

## Discussion

Acute cervical SCI is often associated with cervical fracture, which can lead to serious complications (22). VTE is a common and serious complication after acute SCI. After SCI, dysfunction of the autonomic nervous system leads to lower limb venous vasodilation, increased venous blood flow resistance, decreased endothelial function, weakened platelet inhibition of thrombin production and prostaglandin release, enhanced coagulation function, and relatively weakened fibrinolysis function, resulting in blood being in a hypercoagulable state (10, 23–25). In addition, due to various reasons such as injury itself, limb paralysis after SCI, reduced lower limb activity, prolonged bed rest, surgery, and anesthesia, these are all conducive to the formation of DVT. Many studies have explored the incidence and risk factors of DVT after acute SCI, but mainly focused on the perioperative or postoperative period. They do not pay attention to DVT dynamic change after SCI, or do not comprehensively study the risk factors of DVT exacerbation. To our knowledge, this is the first retrospective study to evaluate the dynamic change of perioperative DVT in patients with SCI complicated with cervical fracture and analyze the risk factors of postoperative DVT exacerbation.

The acute occurrence of DVT is not static, but a dynamic process, which may involve the dissolution and aggravation of thrombus (26, 27). In our study, the majority of thrombi remain unchanged before and after surgery. But about 22.8% of patients experienced postoperative thrombus exacerbation, which was much higher than the patients with thoracolumbar fractures we previously studied (28). This situation may be related to severe muscle pump injury caused by spinal cord injury itself, blood hypercoagulability, and widespread muscle weakness.

In the current study, we found that age, ASIA score, time from injury to surgery, surgery time, and blood loss were risk factors for DVT exacerbation. Many studies found that the older the age, the higher the incidence of DVT in acute SCI patients (13, 15, 29). The results of a study demonstrated increased risk of DVT in acute traumatic SCI who were 50 years of age and older (30). Zhang et al. (15) showed that age had a linearly association with incidence of DVT for patients with SCI, and the incidence of DVT increased by 1.07-fold when age increased by 1 year. Our study found that patients older than 50 years were more likely to have DVT exacerbation after surgery. This is because with the increase of age, the patient's organs are in a state of decline, the blood flow is slow, the blood viscosity is increased, the concentration of clotting factors in the blood vessel endothelial cells is increased, and substances such as antithrombin are reduced, leading to the destruction of the coagulation-anticoagulation balance in the blood system, resulting in the formation of thrombosis (31).

Some studies have shown that loss of motor function is a risk factor for VTE in patients with cervical SCI (9, 10, 13, 22). We also found that ASIA score A-B was a risk factor for postoperative DVT exacerbation. Lower limb paralysis can weaken muscle pump function, reduce blood flow, and promote DVT. One report indicated that SCI above T6 can seriously affect the control of sympathetic nervous system over cardiac function, leading to a decrease in myocardial contractility, dilation of capillaries, gastrointestinal vascular beds, and coronary arteries, resulting in a reduction of approximately 50% in effective blood volume (10). This not only increases blood viscosity, but also greatly reduces muscle oxygen supply and muscle contraction, ultimately leading to a decrease in deep vein blood flow velocity in the lower limbs and an increase in the incidence of DVT (32). Our study found the time from injury to surgery greater than 5 days was closely associated with postoperative DVT exacerbation. Waiting for too long for surgery can prolong the patient's bed rest and braking time. After long-term bed rest, the venous blood flow of the lower extremities slows down or even stops, and the slow blood flow leads to the hypoxia of the valve, causing endothelial damage. Blood stasis can also cause the accumulation of local coagulation factors and the depletion of inhibitory factors, leading to the formation of DVT (10, 33).

We found that surgery time was a risk factor for postoperative DVT exacerbation. A meta-analysis showed that for patients undergoing spinal surgery, prolonging surgical time increased the incidence of DVT (34). This is because surgery can cause tissue factors to enter the blood circulation system, promote the activation of clotting factors, increase blood coagulation degree, but also cause platelet increase, adhesion and aggregation, and ultimately promote the formation and aggravation of DVT. The greater the amount of intraoperative blood loss, the lower the blood volume in the body, resulting in a decrease in blood flow velocity and blood stasis, which increases the risk of DVT. Our study found that increased intraoperative blood loss can lead to DVT exacerbation. We believed that blood loss disrupted the coagulation fibrinolysis system, and that fluid supplementation can modulate the coagulation system, which was consistent with Riha et al. (35). Therefore, by optimizing surgical procedures, improving the skills of surgeons and adequately supplementing fluids, the incidence of DVT exacerbation may be reduced.

The limitations of this study should be acknowledged. Firstly, this retrospective study has inherent limitations. Secondly, the small amount of data result in some variables that may be significantly related to deep vein thrombosis not being recorded or measured. Thirdly, D-dimer is not found to be associated with DVT exacerbation, as our study only included values at admission. Future prospective, large sample and multi-center studies are needed to confirm our results.

## Conclusions

In patients with spinal cord injury complicated with cervical fracture, the risk of postoperative DVT exacerbation is very high. Age, ASIA score A-B, time from injury to surgery, surgery time and blood loss were risk factors for postoperative DVT exacerbation or new DVT.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Author contributions

HW: Writing – original draft, Writing – review & editing. BL: Conceptualization, Writing – review & editing. WL: Conceptualization, Writing – review & editing. JX: Writing – original draft, Writing – review & editing. CM: Writing – original draft, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



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# The impact of inflammatory and oxidative stress biomarkers on the sympathetic nervous system in severe coronary atherosclerosis

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**Objective:** The present study aimed at evaluating the association between sympathetic nervous system activation (SNS) and the severity of coronary artery disease (CAD). In addition, we tested the hypothesis that inflammation and oxidative stress influence the SNS activation.
**Methods:** Adult patients with severe CAD scheduled for coronary artery bypass graft (CABG) surgery were enrolled. SYNTAX I score was calculated based on coronary angiography. Systemic activation of the SNS was estimated through circulating levels of norepinephrine (NE). Plasma levels of pro-inflammatory cytokines (IL 1β, IL 6 and HIF 1α) and oxidative stress molecules (SOD-1 and LOX-1) were obtained prior to surgery.
**Results:** Circulating NE levels were significantly correlated with the severity of CAD, as assessed by the SYNTAX I score ($p$ 0.002; $r$ 0.329). Elevated levels of circulating pro-inflammatory markers were significantly correlated with increased NE concentrations (for IL-1β: $p < 0.001$, $r = 0.49$; for IL-6 and NE: $p = 0.003$, $r = 0.32$; for HIF-1α and NE: $p = 0.049$, $r = 0.21$). Additionally, oxidative stress molecules were associated with circulating NE levels (for SOD-1 and NE: $p = 0.016$, $r = 0.26$; for LOX-1 and NE: $p = 0.004$, $r = 0.31$).
**Conclusion:** In patients with CAD referred for CABG, SNS activation, indicated by plasma NE levels, was correlated with disease severity as assessed by the SYNTAX I score, as well as with markers of inflammation and oxidative stress. This suggests that inflammation, oxidative stress, and SNS activation form an interconnected network, with each component influencing the others. It might be of interest to develop a scoring system including inflammation and oxidative stress markers to identify patients that require a more aggressive approach to lower inflammation, oxidative stress and modulate the sympathetic nervous system. This could be of use especially in the setting of a scheduled intervention -such as CABG surgery.

KEYWORDS

**inflammation, coronary artery disease, oxidative stress, sympathetic nervous system, coronary artery bypass graft**

Abbreviations
ANS, autonomic nervous system; BMI, body mass index; CABG, coronary artery bypass graft; CAD, coronary artery disease; ELISA, enzyme-linked immunosorbent assay; HIF 1 alpha, hypoxia-inducible factor 1-alpha; HRV, heart rate variability; IL-1, interleukine-1; IL-6, interleukine-6; LOX 1, lectin-like oxidized low-density lipoprotein receptor-1; NADPH, nicotinamide adenine dinucleotide phosphate; NE, norepinephrine; Ox-LDL, oxidized low-density lipoprotein cholesterol; ROS, reactive oxygen species; SD, standard deviation; SOD-1, superoxide dismutase-1; SNS, sympathetic nervous system; SYNTAX I, synergy between percutaneous coronary intervention with taxus and cardiac surgery.

# 1 Introduction

Inflammation plays a pivotal role in all stages of atherosclerosis, from subclinical endothelial dysfunction to severe atheroma burden and acute cardiovascular events (1). The interleukine-1 (IL-1) family is a major cytokine family (to date 11 members have been discovered; initially comprising only two forms, IL 1 α and IL 1 β) an associated with various cardiovascular diseases (2). Interleukine-6 (IL-6), also an important marker of inflammation, is independently associated with major adverse cardiovascular disease (2, 3). Previous studies have shown that injections of IL 1 and IL 6, pro inflammatory cytokines, cause an activation of the SNS, with release of norepinephrine (NE) from the central as well as the peripheral SNS (4). Hypoxic areas in atheroma plaques lead to the expression of hypoxia-inducible factor 1-alpha (HIF 1 α). HIF 1 α influences cellular functions and cytokine expression inmacrophages, vascular smooth muscle cells and endothelial cells, key elements of the atherosclerotic process (5). Oxidative stress contributes to the development of coronary artery disease (6), lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1), of the scavenger receptors for oxidized low-density lipoprotein cholesterol (ox-LDL)- and superoxide dismutase-1 (SOD 1) being crucial components of the process. Oxidative modifications of low-density lipoprotein to oxidized LDL play an important role in the initiation and progression of atherosclerosis. LOX-1 was identified as the major oxidized LDL receptor in endothelial cells (7). SOD 1 (along with other enzymes in the SOD family) protects cells by scavenging harmful superoxide radicals. SOD is an important marker of lipid peroxidation and of the progression of atherosclerosis correlated with oxidative stress (8).

In ischemic cardiomyopathy aberrant remodeling of the SNS occurs, with effects mainly modulated by NE. Histologic changes in stellate ganglia neurons have been observed resulting in inflammation, glial cell activation, and oxidative stress (9, 10).

Growing evidence suggests that the autonomic nervous system (ANS) plays a crucial role in regulating systemic inflammation, and an imbalance in the ANS may elevate the risk of acute cardiovascular events by promoting inflammation and damaging the endothelium (11).

Clinical studies have previously demonstrated an inverse correlation between SNS activation, assessed through heart rate variability, and chronic low-grade systemic inflammation in patients with stable CAD (12). However, it remains unclear whether SNS activation and inflammation are directly associated with the severity of CAD.

This study aimed to evaluate the association between norepinephrine (NE), as a surrogate marker of sympathetic nervous system (SNS) activation, and the severity of coronary artery disease (CAD), as determined by the SYNTAX score. Additionally, it sought to investigate potential correlations between inflammation, measured by IL-1β, IL-6, and HIF-1α, oxidative stress mediators, such as LOX-1 and SOD-1, and SNS activation, assessed through plasma NE levels, in patients with severe CAD who are candidates for surgical revascularization.

# 2 Methods

## 2.1 Design, study population

This study prospectively included 84 adult patients with severe CAD scheduled for CABG surgery, between January 2020 and June 2021. The study protocol obtained ethical clearance from the Ethical Committee of Transylvania University (registration number 1/2.03.2019) and adhered to the principles outlined in the Helsinki Declaration and the Code for Good Clinical Practice. A written Informed Consent was obtained from all patients.

Patients with acute coronary syndromes, significant valvular disease, severe hepatic or renal failure, recent or active bleeding, coagulation disorders, active malignancy, and inflammatory diseases (including infections and autoimmune disorders) were excluded from the study. Additionally, patients diagnosed with pheochromocytoma or those undergoing psychiatric treatment (such as with serotonin-norepinephrine reuptake inhibitors) were excluded due to the potential impact on norepinephrine (NE) levels. Patients taking alpha-2 blockers were also not included, and alpha agonists were not administered during anesthesia.

At admission, all patients underwent clinical, echocardiographic, and coronary angiography evaluations, and blood samples were collected in accordance with the clinic's protocol. Based on coronary angiogram, the SYNTAX I score was determined using the number of diseased arteries, the location, and the aspect of atherosclerotic plaques (https://syntaxscore.org/).

## 2.2 Measurement of biomarkers

Peripheral venous blood samples were drawn after a minimum of 8 h fasting, 3 days prior CABG. Samples were centrifuged and supernatant was frozen at −80°C until the final measurements. HIF 1 α, IL 1β, IL 6, LOX-1, and NE concentrations were determined by enzyme-linked immunosorbent assay (ELISA) and SOD-1 concentrations were measured using colorimetric determinations, with commercially available kits (Elabscience Biotechnology Inc., Houston, Texas, United States) in accordance with the manufacturer's instructions. All measurements were completed by the same technician who had no access to clinical information.

## 2.3 Statistical analysis

Categorical variables were expressed as *n* (%), normally distributed data and skewed data of continuous variables were expressed as mean ± standard deviation (SD), and median (minimum-maximum), respectively. The normality of continuous variables was tested by Shapiro-Wilk's test. To ascertain distinctions in the analyzed data, 2-tailed Pearson correlation (for variables with normal distribution) and Spearman's correlation

(for variables with skewed distribution) were applied. Statistical significance was set at $p < 0.05$. Analysis was performed using Microsoft Excel 2007 and JASP 0.19 software.

## 3 Results

The study population consisted of 84 patients aged 46–88 (mean age: 65), of which 67 were males. The mean body mass index (BMI) was 28.36 kg/m$^2$. The documented cardiovascular risk factors included smoking history (20.23%), dyslipidemia (95.23%), hypertension (94.04%), and diabetes mellitus (39.28%). Patients' characteristics and laboratory test findings are presented in Table 1.

TABLE 1 Patients' characteristics.

| Variables | |
|---|---|
| Age (years) mean ± SD | 65 ± 8.09 |
| Male gender *n*, (%) | 67 (79.76) |
| BMI (kg/m$^2$) mean ± SD | 28.36 ± 4.24 |
| Cardiovascular risk factors *n*, (%) | |
| Smoking history | 17 (20.23) |
| Dyslipidemia | 80 (95.23) |
| Hypertension | 79 (94.04) |
| Diabetes mellitus | 33 (39.28) |
| SYNTAX score median (min, max) | 29.75 (17, 54) |
| Laboratory data | |
| IL 1 β (pg/ml) mean ± SD | 8.41 ± 1.92 |
| IL 6 (pg/ml) mean ± SD | 80.13 ± 10.07 |
| HIF 1 α (pg/ml) median, (min-max) | 201.10 (55.54–585.91) |
| LOX 1 (pg/ml) median, (min-max) | 1,191.54 (789.0–2,457.90) |
| SOD 1 (ng/ml)mean ± SD | 1.37 ± 0.23 |
| NE (pg/ml) median, (min-max) | 2,964.18 (1,356.89–3,622.62) |

BMI, body mass index; SD, standard deviation; IL 1β, interleukine 1β; IL 6, interleukine 6; HIF 1 α, hypoxia-inducible factor 1-alpha; LOX-1, lectin-like oxidized low-density lipoprotein receptor-1; SOD 1, superoxide dismutase-1; NE, norepinephrine.

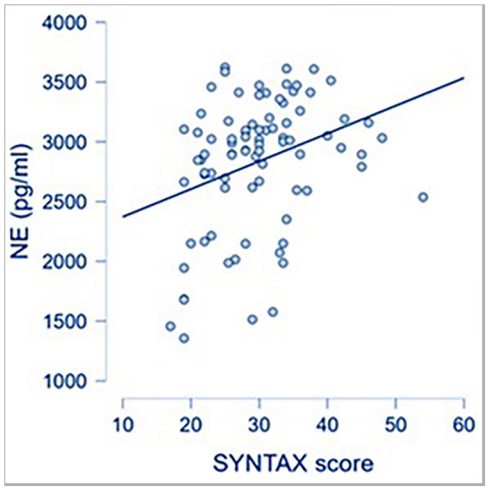


FIGURE 1
Correlation between SYNTAX score and NE ($p = 0.002$; $r = 0.329$).

CAD severity, as reflected by a high SYNTAX score, was associated with increased systemic levels of NE, used as a surrogate for SNS activation (Figure 1).

The correlations between each inflammatory parameter and norepinephrine (NE) are shown in Figure 2. Statistically significant correlations were observed between IL-1β and NE, IL-6 and NE, as well as HIF-1α and NE.

Circulating levels of oxidative stress markers (SOD-1 and LOX-1) were also correlated with circulating NE at a statistically significant level, as presented in Figure 3.

The analysis conducted to identify potential confounding factors found that none of the cardiovascular risk factors, including BMI, smoking, dyslipidemia, hypertension, and diabetes mellitus, was independently associated with NE levels, nor with the inflammatory markers (IL-1β, IL-6, HIF-1α) or oxidative stress indicators (SOD-1 and LOX-1) (see Supplementary Tables S1–S6 in the Supplementary Material).

## 4 Discussion

CAD is a disease state characterized by inflammation, increased oxidative stress and overstimulation of the SNS. Because these 3 aspects are interconnected, modulating any of them might prove useful for reducing the atherosclerotic burden and might, as well, have beneficial effects on the other components of this intricate network.

IL 1β plays a central role in local inflammation, being produced by monocytes/macrophages engulfed in the arterial wall in atherosclerosis. IL 1β mediates the production of IL 6, which in turn acts systemically activating more inflammatory cytokines and acute phase response proteins (13). Loss of viable myocardium in the setting of myocardial infarction is attributed to reperfusion injury and inflammatory response. Several recent trials have regarded pro-inflammatory cytokines as therapeutic targets, using specific antibodies in patients presenting with myocardial infarction. The largest of them, the CANTOS trial (Canakinumab Anti-inflammatory Thrombosis Outcome Study), which used Canakinumab, a monoclonal antibody binding selectively to IL 1β, enrolled patients with established CAD and residual inflammation. Its results proved a significant decrease of cardiovascular and cerebral events and cardiovascular death (14). However, the use of Tocilizumab, antibody targeting IL 6, did not show a significant clinical benefit in research conducted thus far (15, 16). An elevated pre-CABG surgery level of IL 6 has been associated with early graft occlusion and late cardiovascular events (17).

HIF 1 α is another molecule of interest, as it has links with both inflammation and oxidative stress. HIF 1 α stimulates macrophage activation, leading to lipid uptake and inflammation (including IL 1 β induction) and is also associated with reactive oxygen species (ROS) production by endothelial cells, activating NADPH oxidase genes (18). Also, HIF 1 α induces the expression of LOX 1 receptor at the cell surface, thus enhancing lipid uptake in macrophages (19). The HIF pathway remains of interest especially as its modulation could limit the effects of ischemia/

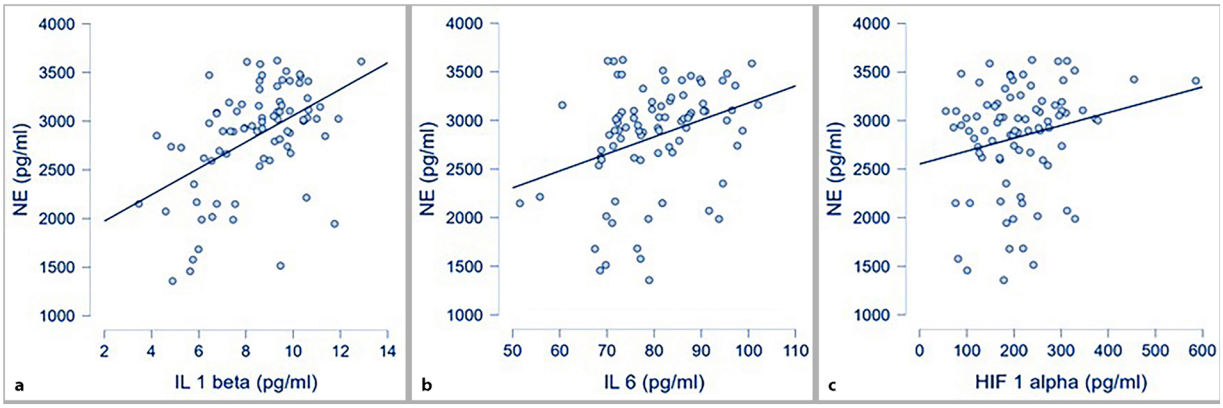


FIGURE 2
Correlations between inflammatory markers and NE; **(a)** IL 1 β and NE ($p < .001$; $r$ 0.49); **(b)** IL 6 and NE ($p = 0.003$; $r$ 0.32); **(c)** HIF 1α and NE ($p$ 0.049; $r$ 0.21).

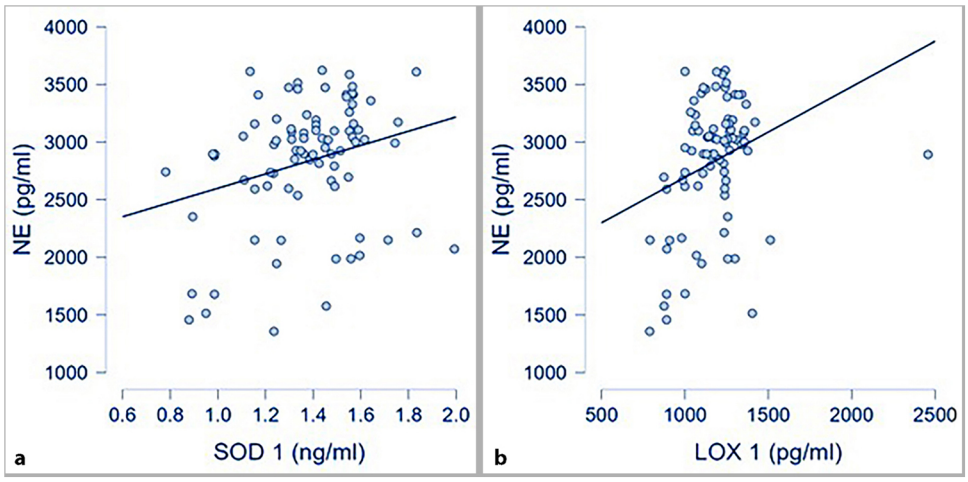


FIGURE 3
Oxidative stress markers and NE; **(a)** correlation between SOD-1 and NE ($p$ 0.016; $r$ 0.26); **(b)*** correlation between LOX-1 and NE ($p$ .004; $r$ 0.31; *spearman correlation for skewed distributed variables).

reperfusion (for example in the of CABG surgery) or it could be used for ischemic pre-/post-conditioning, thus limiting the magnitude of an acute coronary event (20). However, recent research shows that maintaining an HIF 1 α intracellular homeostasis rather than totally inhibiting its effects could be the optimal approach to managing this important signaling pathway. HIF target-genes (such as adenosine receptors) could be activated directly. Some of these approaches may lead to novel pharmacologic strategies to prevent or treat organ injury in surgical patients (21). A recent trial showed that spironolactone is also involved in reducing HIF 1 α levels in patients undergoing CABG surgery (22).

SOD enzymes-SOD 1 being the major intracellular SOD- are part of the antioxidant defense system, implicated in lipid peroxidation and progression of atherosclerosis correlated with oxidative stress (23). However, in cases of increased SOD activity, such as in states of ischemia/reperfusion, the beneficial antioxidant effects are lost, increased damaged to the cell ensues (6, 8). CABG surgery induces increased levels of oxidative stress; hence the SOD system might be an efficient antioxidant (24).

Furthermore, increased ROS production also leads to the oxidation of native LDL to oxidized LDL (ox-LDL), which contributes to atherosclerotic plaque formation and progression by exerting its effects through its receptor-LOX-1 (25). LOX-1 expression is induced by several molecules: inflammatory cytokines (such as IL-1 and IL-6), angiotensin II, sheer stress, and glycation end-products. In turn, LOX-1 amplifies ROS formation by increasing NADPH oxidase activity. LOX-1 levels are upregulated in the heart after an

ischemia/reperfusion event (CABG surgery), making it a potential target for therapy (26, 27).

Our study used a unique combination of biomarkers of inflammation (IL 1 beta, Il 6 and HIF 1 alpha) and oxidative stress (SOD 1 and LOX 1), each studied parameter proving to be correlated with increased levels of NE, neurohormone used to assess the systemic activity of the SNS.

To our knowledge, this is the first study to evaluate this combination of serum markers in patients with severe coronary artery disease (CAD) scheduled for surgical revascularization—a procedure that exposes patients to significant ischemia/reperfusion. The study demonstrates a statistical correlation between these markers and plasma norepinephrine (NE) levels, which serve as a surrogate for sympathetic nervous system (SNS) activation.

Recent research has proven that SNS is intimately associated with inflammation and oxidative stress (28, 29). Stimulation of central NE release also increases peripheral plasma NE: experimental studies conducted so far have shown that IL-1β and IL-6 injections determine short-lasting increases of NE (30, 31). In contrast, our study found high NE plasma concentrations correlated with high systemic levels of IL-1 β and IL-6, most likely a chronic state in patients with severe CAD.

Autonomic dysregulation is present throughout cardiovascular disease, with the interest in neuromodulation remaining increased. Previous research has proven that remodeling of the autonomic nervous system and the intrinsic cardiac nervous system occurs in various disease states, including the setting of myocardial infarction (32). NE exerts its effects on α and β adrenoceptors. α1 blockers exert anti-atherosclerotic effects by lowering blood pressure and modifying lipid profile (33). α2 adrenoceptors agonists also hold anti-atherosclerotic properties, possibly by reducing inflammation and stimulating oxidized LDL clearance, as recent animal studies show (34). Beta (β1, β2 and β3) blockers inhibit atherosclerosis progression by reducing circulating levels of proinflammatory cytokines, inhibiting oxidative stress, preventing LDL oxidation, decreasing monocyte adhesion to endothelium, and macrophage content in the atheroma plaque, conclusions drawn from experimental studies (35–37). However, the consequences of blunting the effect of SNS by renal denervation on atherosclerosis require further research, as present studies demonstrated that the procedure actually haslimiting adverse effects (38).

Recent research has proven that ischemic cardiomyopathy is accompanied by remodeling of the intrinsic cardiac nervous system, stellate ganglia, and higher nervous centers (39). Alterations of neurohumoral control and circulating catecholamines also occur (40). There is increasing evidence of the crosstalk between the immune system and the SNS (41). Cytokines stimulate the activation of SNS, which is further integrated at the hypothalamic level. In turn, NE can modulate the functions of the immune system.

Various markers of inflammation and oxidative stress work as a network and it is difficult to make associations between only one or few of them with cardiovascular risk and outcomes.

Moreover, excessive release of ROS can lead to cellular damage, negatively impacting not only the cardiovascular system, but also other bodily systems. Recent evidence suggests that oxidative stress plays a role in various neurodegenerative and neuropsychiatric disorders (42, 43).

## 5 Future directions

The prognostic value of a polyparametric approach was demonstrated in the setting of acute coronary syndromes, with the development of an Inflammatory Score, which unfortunately has not been further evaluated in other populations (44). It might be of interest to evaluate the prognostic value of a scoring system (including pro inflammatory markers, oxidative stress markers and NE) in the setting of ischemia/reperfusion injury (such as that induced by CABG). Furthermore, the use of targeted single cell treatments might be of interest in the management of selected patients (45).

## 6 Study limitations

Several limitations of the study must be mentioned. Firstly, the cytokines and oxidative stress markers that were tested are only a few of the molecules involved in the atherosclerotic process. Secondly, we only measured their circulating levels at one-point, serial determinations might be more suitable for establishing solid correlations.

## 7 Conclusion

In patients with CAD referred for CABG, SNS activation, as indicated by plasma NE levels, was correlated with disease severity, assessed by the SYNTAX I score. Additionally, NE levels were associated with markers of inflammation and oxidative stress, suggesting that inflammation, oxidative stress, and SNS activation form an interconnected network, where each component influences the others. It may be valuable to develop a scoring system that incorporates inflammation and oxidative stress markers to better identify patients who require a more aggressive strategy to reduce inflammation, oxidative stress, and modulate sympathetic nervous system (SNS) activity. Such a system could be particularly useful in the context of scheduled interventions, such as coronary artery bypass grafting (CABG) surgery, to tailor treatment and improve outcomes.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Ethics statement

The studies involving humans were approved by Ethical Board of the Transylvania University. The studies were conducted in

accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

## Author contributions

AB: Data curation, Formal Analysis, Investigation, Methodology, Project administration, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. CDL: Writing – review & editing, Data curation, Visualization. CN: Writing – review & editing, Data curation, Formal analysis, Investigation. DȚ: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2024.1480925/full#supplementary-material

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# Cardioprotection in coronary artery bypass graft surgery: the impact of remote ischemic preconditioning on modulating LOX-1 and SOD-1 to counteract oxidative stress

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**Background:** Coronary artery bypass grafting (CABG) is frequently used to treat severe coronary artery disease (CAD), but it can lead to increased oxidative stress and inflammation, worsening patient outcomes. Remote ischemic preconditioning (RIPC) has been suggested as a potential strategy to protect against these effects by modulating oxidative stress and inflammatory responses, though its impact on specific biomarkers requires further investigation. This study aims to assess the effects of remote ischemic preconditioning on inflammation markers and oxidative stress in patients with severe CAD undergoing coronary artery bypass grafting.

**Methods:** We conducted a case-control study involving 80 patients with severe coronary artery disease (CAD) scheduled for coronary artery bypass grafting (CABG). Fifty percent of these patients received ischemic preconditioning prior to surgery. Plasma levels of Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) and Superoxide dismutase-1 (SOD-1) levels were measured in all individuals using the ELISA method at three important time points: before surgery (visit 1 or V1), immediately post-operatively (visit 2 or V2), and one week post-operatively (visit 3 or V3).

**Results:** We enrolled 80 patients, of which 40 were assigned to the studied group receiving remote ischemic preconditioning (RIPC) and 40 to the control group. There were no statistically significant differences between the groups regarding baseline, clinical, or operative characteristics. RIPC treatment significantly reduced plasma levels of Lectin-like oxidized low-density lipoprotein (LDL) receptor-1 (LOX-1) ($p < 0.05$) as well as significantly increasing total values of Superoxide dismutase-1 (SOD-1) ($p < 0.05$, respectively). There were notable differences between the studied and control groups at V2 and V3. The studied group had higher SOD-1 levels ($p < 0.05$) and significantly lower LOX-1 levels at both time points ($p < 0.05$).

**Conclusion:** The significant changes in plasma levels of both LOX-1 and SOD-1 observed in this study strongly suggest that remote ischemic preconditioning (RIPC) plays an important role in reducing oxidative stress and enhancing the antioxidative status of patients. This is evidenced by the marked decrease in LOX-1 levels, alongside a corresponding increase in SOD-1 levels, indicating that RIPC may contribute to improved cardioprotection through these mechanisms.

KEYWORDS

remote ischemic preconditioning, cardioprotection, LOX-1, SOD-1, oxidative stress, coronary artery bypass graft surgery

# 1 Introduction

Remote Ischemic Preconditioning (RIPC) stands as a noninvasive approach designed to protect the heart and other organs from the harmful effects of lethal ischemia and reperfusion injury. The method involves brief cycles of limb ischemia and reperfusion, typically carried out by alternately inflating and deflating a blood pressure cuff on one or more limbs to a suprasystolic pressure value for several cycles (1).

When organs are repeatedly exposed to short-term ischemia-reperfusion, less damage occurs during final reperfusion compared to prolonged ischemic episodes, for example in patients undergoing coronary artery bypass grafting (CABG) and remote ischemic conditioning (RIC) (2, 3).

This method was first described by Murry et al. in 1986 in a study on the heart of dogs (4), which demonstrated the adaptability of the heart to ischemic episodes lasting several minutes, a phenomenon termed ischemic preconditioning (IP) (5, 6).

In humans, Przyklenk et al. described the RIPC phenomena for the first time at the end of the twentieth century (7). After several years, the concept of "cardio protection at a distance" through ischemia conditioning was soon expanded to other tissues and organs, as well as to greater distances from the heart (8). The underlying mechanisms are likely to include transferable humoral release from perfused tissue as well as neuronal responses (9).

Despite myocardial preservation strategies, coronary artery bypass grafting (CABG) is still associated with severe complications.

During CABG, the blood flow to the heart and lungs is temporarily interrupted. Lack of oxygen in these organs disrupts the balance between oxidant and antioxidant enzymes through inefficient degradation of free oxygen radicals, increased/reduced or abnormal activity of antioxidant enzymes, triggering oxidative stress through the accumulation of reactive oxygen species (ROS) (10–12).

Antioxidants are molecules that can inhibit the oxidation of other molecules by destroying oxidants or decreasing the production of free oxygen radicals. The major antioxidant systems present in vascular walls include Superoxide Dismutase (SOD), Catalase, Glutathione Peroxidase, Thioredoxin and Peroxiredoxin (11, 12).

Low-density oxidized lipoprotein receptor type 1 (LOX-1) plays a critical role in the production of atherosclerosis. LOX-1, in addition to binding and internalizing ox-LDL, also contributes to endothelial dysfunction and apoptosis, aiding foam cell formation in macrophages and smooth muscle cells and platelet activation (13).

Remote ischemic preconditioning (RIPC) has been reported to reduce reperfusion injury in people undergoing cardiac surgery and improve clinical outcome (14).

While remote ischemic preconditioning (RIPC) has attracted interest for its potential cardioprotective effects, particularly in patients undergoing cardiac or vascular surgeries, its use is not free from possible complications. Commonly regarded as a non-invasive and low-risk intervention, RIPC may still present challenges in certain patient populations. One possible complication is transient discomfort or pain during the ischemic episodes, which may discourage adherence in some patients (15). Furthermore, there have been reports of hypotension following RIPC, particularly in patients with pre-existing cardiovascular instability (16). This hypotensive response may be more pronounced in patients during general anesthesia or in those with compromised cardiovascular function (17).

Another significant concern is the variability in the efficacy of RIPC, which can be influenced by factors such as age, comorbidities, and medications (especially antihypertensive drugs) (18). For instance, patients with diabetes or hypertension may exhibit an attenuated response to RIPC, possibly due to underlying endothelial dysfunction (19). This reduced responsiveness could limit the protective benefits of the procedure and may increase the risk of ischemic events. Lastly, recent studies have suggested that repetitive RIPC may lead to paradoxical effects, such as exacerbation of ischemia-reperfusion injury in some cases, although this remains an area of ongoing investigation (20).

This study aims to assess the effects of remote ischemic preconditioning on inflammation markers and oxidative stress in patients with severe CAD undergoing coronary artery bypass grafting.

# 2 Materials and methods

## 2.1 Patients

We conducted a prospective observational study, consecutively enrolling 80 adult patients (over 18 years) with severe coronary artery disease who were indicated for elective CABG and consented to undergo the surgery (Figure 1).

The inclusion criteria for this study stipulated that participants must have a body weight greater than 50 kg, be over the age of

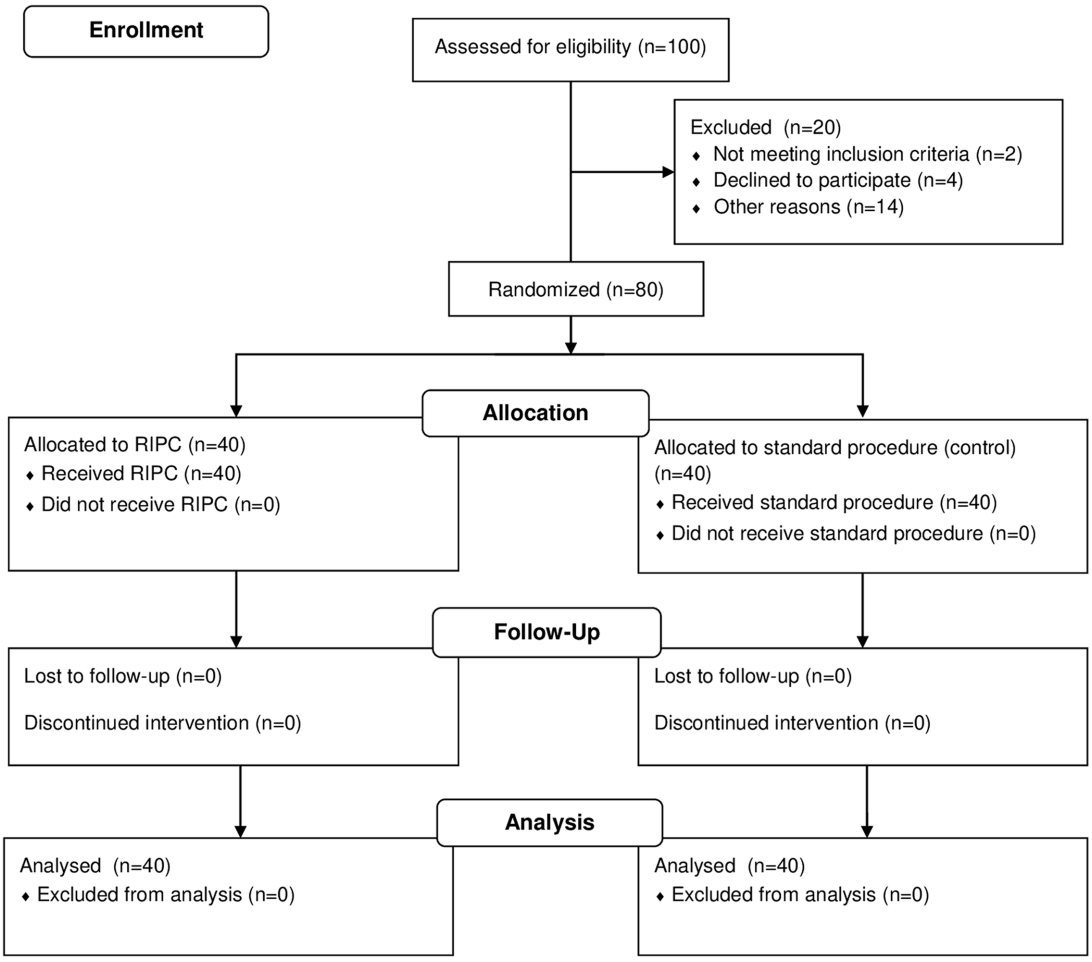


FIGURE 1
Randomization and follow-up flowchart.

18 years, and have provided written informed consent, appropriately dated and signed, prior to their inclusion in the study. Patients with a history of prior revascularization, decompensated heart failure, left ventricular ejection fraction (LVEF) less than 30%, severe renal failure (grade 4 or on dialysis), hepatic dysfunction (Child-Pugh class B or higher), severe pulmonary disease, indication for emergency CABG, and those unable or unwilling to provide informed consent were excluded.

After inclusion, the patients were randomly assigned in a 1:1 ratio to either undergo RIPC or be part of the control group. All members responsible for conducting the study were blinded from the RIPC procedure.

The research was carried out at Clinicco Hospital in Brasov, from January 2020 to November 2022. All procedures in this study were conducted in accordance with the Helsinki Declaration for research involving human subjects and obtained ethical approval from the Ethics Review Board of Transilvania University of Brașov.

Upon admission, patients underwent an initial evaluation comprising their past medical history and present clinical condition, alongside assessments from physical examinations, echocardiography, and laboratory tests.The severity of coronary lesions was quantified using the Syntax score, based on the coronary angiography previously performed in all patients, approximately 2 to 4 weeks prior to the index hospitalization for CABG.

## 2.2 Blood sample collection and analysis

Blood samples for blood cell counts, glycemia, renal function, hepatic function, cardiac biomarkers such as creatin-kinase MB (CK-MB), high sensitivity troponin I (HSTnI), other inflammatory markers and other biological parameters were collected per hospital protocol.

For the specific parameters in our study, we processed the samples by centrifugation to separate the serum from the plasma, which were then labeled and stored at −80 degrees Celsius. We assessed LOX-1 and SOD-1 using the ELISA test in both preconditioned and non-preconditioned populations at three key moments: before surgery (visit 1 or V1), immediately post-operatively (visit 2 or V2), and one week post-operatively (visit 3 or V3).

## 2.3 RIPC procedure

The procedure of RIPC was carried out on the day of surgery, before the induction of anesthesia. It consisted of inflating a blood pressure cuff on the upper and lower limbs to 200 mmHg for 5 min (ischemia stage), followed by a 5-minute time-out with the cuff deflated (reperfusion stage) for a total number of 4 cycles.

Anesthetic management, cardiopulmonary bypass, cardioplegia, surgical techniques, and all other aspects of pre- and postoperative management adhered to the existing protocols in the hospital. All patients received the same types of general anesthesia, including volatile inhalation and intravenous hypnotics.

## 2.4 Statistical analysis

The database has been created using Microsoft Excel 2019 and values have been interpreted by JASP 0.19.0. Categorical variables were given as counts or absolute frequencies. To observe the difference between the mean values of two variables we used Student's *t*-test or Mann-Whitney U (normal distribution or not), and values of $p < 0.05$ were considered statistically significant. *post hoc* analyses, in ANOVA RM, determined the differences within and between groups.

The parameters for the power analysis included an expected effect size of Cohen's $d = 0.5$ for a medium effect, a significance level (alpha) of 0.05, and a target power of 0.80. This power level was chosen to provide an 80% probability of detecting a true effect, if it exists, thereby balancing the risk of Type I (false positive) and Type II (false negative) errors.

# 3 Results

Of the 100 patients who underwent screening, 80 were successfully enrolled in the study, as depicted in Figure 1. The remaining 20 patients were not included due to the following reasons: 2 patients did not meet the inclusion criteria of a weight above 50 kg, 4 participants declined enrollment in the study due to their unwillingness to sign the informed consent form, and 14 patients were excluded from the study for various reasons, as documented in the CONSORT flow chart: 2 patients presented with severe renal impairment, 2 had a history of prior revascularization, 4 were diagnosed with decompensated heart failure, and 6 faced logistical challenges such as transportation difficulties or residing at a considerable distance from the study site.

From the total of 80 patients that were enrolled, 40 were randomized to receive RIPC, and 40 were allocated to the control group. There were no losses to follow-up, and no patients were prematurely excluded from the study. Additionally, no complications or adverse events were observed during the application of RIPC.

The baseline characteristics of the patients in both the studied and control groups were generally well-matched, with no statistically significant differences observed, as demonstrated in Table 1. The mean age of participants was comparable between the studied group (65.00 ± 7.57 years) and the control group (64.47 ± 8.53 years, $p = 0.90$). Gender distribution also showed a slight difference, with the studied group having a higher proportion of males (85%) compared to the control group (75%), but the difference was not statistically significant. The mean body mass index (BMI) and the smoking status were similar between groups.

The clinical characteristics of the patients were generally comparable between the studied and control groups, as illustrated in Table 2. Dyslipidemia was present in all patients in the studied group (100%) compared to 92.5% in the control group ($p = 0.10$). The prevalence of arterial hypertension (HTN) was high in both groups, with 97.5% in the studied group and 92.5% in the control group ($p = 0.21$). Type 2 diabetes mellitus (T2DM) was observed in 40% of the studied group and 37.5% of the control group ($p = 0.36$). A history of myocardial infarction (MI) was noted in 30% of the studied group and 42.5% of the control group ($p = 0.11$), while a history of stroke was identical in both groups at 5% ($p = 0.98$). Peripheral artery disease (PAD) was slightly more prevalent in the control group (15%) compared to the studied group (7.5%), though this difference did not reach statistical significance ($p = 0.10$).

Overall, the baseline characteristics and clinical profiles of the patients were well-balanced between the two groups, ensuring that the study's outcomes were not influenced by disparities in these characteristics.

The operative characteristics of the patients exhibited no significant differences between the studied and control groups, suggesting comparable surgical experiences and outcomes across both cohorts, which were not influenced by RIPC, as depicted in as depicted in Table 3. The SYNTAX I score, which assesses the complexity of coronary artery disease, was comparable between the studied group (29.58 ± 7.94) and the control group (30.51 ± 8.59, $p = 0.61$). Aortic clamp time (ACT) and cardiopulmonary bypass time (CPBT) were also similar, with the studied group

TABLE 1 Baseline characteristics of the patients.

| | Studied group $N = 40$ | Control group $N = 40$ | *p* |
|---|---|---|---|
| Age—years (mean) ±SD | 65 ± 7.57 | 64.47 ± 8.53 | 0.90 |
| Gender (male)—*n*, (%) | 34, (85) | 29, (72.50) | 0.08 |
| BMI—kg/m$^2$ ± SD | 27.88 ± 4.26 | 29.36 ± 3.93 | 0.98 |
| Smoking—*n*, (%) | 9, (22.50) | 6, (15) | 0.08 |

TABLE 2 Clinical characteristics of the patients.

| | Studied group $N = 40$ | Control group $N = 40$ | *p* |
|---|---|---|---|
| Dyslipidemia *n*, (%) | 40, (100.00) | 37, (92.50) | 0.10 |
| HTN *n*, (%) | 39, (97.50) | 37, (92.50) | 0.21 |
| T2DM *n*, (%) | 16, (40.00) | 15, (37.50) | 0.36 |
| Old MI *n*, (%) | 12, (30.00) | 17, (42.50) | 0.11 |
| History of stroke *n*, (%) | 2, (5.00) | 2, (5.00) | 0.98 |
| PAD *n*, (%) | 4, (7.50) | 8, (15) | 0,10 |

HTN, arterial hypertension; T2DM, type 2 diabetes mellitus; MI, myocardial infarction; PAD, peripheral artery disease.

TABLE 3 Operative characteristics of the patients.

| | Studied group $N = 40$ | Control group $N = 40$ | $p$ |
|---|---|---|---|
| SYNTAX I—$n \pm$ SD | 29.58 ± 7.94 | 30.51 ± 8.59 | 0.61 |
| ACT (min)—$n \pm$ SD | 65.45 ± 28.20 | 76.42 ± 26.33 | 0.97 |
| CPBT (min)—$n \pm$ SD | 81.20 ± 28 | 93.50 ± 28.31 | 0.97 |
| LVEF/pre (%)—$n \pm$ SD | 51.75 ± 7.97 | 48.37 ± 9.63 | 0.89 |
| LVEF/post (%)—$n \pm$ SD | 51.62 ± 7.37 | 49.37 ± 8.02 | 0.90 |
| ICU days ($n$)—$n \pm$ SD | 3.62 ± 0.86 | 4.10 ± 1.59 | 0,97 |
| Hosp days ($n$)—$n \pm$ SD | 10.30 ± 2.51 | 10.55 ± 3.17 | 0.98 |

ACT, aortic clamp time; CPBT, cardio-pulmonary bypass time; LVEF/pre, left ventricular ejection fraction preoperatory; LVEF/post, left ventricular ejection fraction postoperatory; ICU, intensive care unit.

having an ACT of 65.45 ± 28.20 min compared to 76.42 ± 26.33 min in the control group ($p = 0.97$), and a CPBT of 81.20 ± 28.00 min vs. 93.50 ± 28.31 min ($p = 0.97$). Preoperative left ventricular ejection fraction (LVEF) was slightly higher in the studied group (51.75 ± 7.97%) compared to the control group (48.37 ± 9.63%, $p = 0.89$), with similar postoperative LVEF values (51.62 ± 7.37% vs. 49.37 ± 8.02%, $p = 0.90$). The number of days spent in the intensive care unit (ICU) and the total hospital stay were also closely matched, with ICU stays averaging 3.62 ± 0.86 days in the studied group and 4.10 ± 1.59 days in the control group ($p = 0.97$), and total hospital stays of 10.30 ± 2.51 days and 10.55 ± 3.17 days, respectively ($p = 0.98$).

The general laboratory tests revealed no statistically significant differences between the studied and control groups across all measured parameters, as demonstrated in Table 4 and Figure 2.

Hemoglobin levels (Hb) before surgery were similar between the studied group (13.66 ± 1.44 g/dl) and the control group (13.56 ± 1.19 g/dl, $p = 0.73$), as were levels of C-reactive protein (CRP) (0.36 ± 0.43 mg/dl vs. 0.80 ± 1.60 mg/dl, $p = 0.10$) and high-sensitive troponin I (HSTnI) (0.039 ± 0.076 ng/ml vs. 0.05 ± 0.12 ng/ml, $p = 0.57$).

Serum creatinine levels also showed no significant difference preoperatively between the studied (0.99 ± 0.300 mg/dl) and control groups (0.97 ± 0.30 mg/dl, $p = 0.83$).

Similar trends were observed postoperatively, with Hb (10.23 ± 1.24 g/dl vs. 10.14 ± 1.00 g/dl, $p = 0.71$), CRP (6.35 ± 2.69 mg/dl vs. 6.43 ± 3.47 mg/dl, $p = 0.90$), HSTnI (0.67 ± 0.69 ng/ml vs. 1.22 ± 3.48 ng/ml, $p = 0.33$), and serum creatinine (1.03 ± 0.35 mg/dl vs. 1.10 ± 0.47 mg/dl, $p = 0.43$) with plasma levels remaining comparable between the groups.

Finally, the follow-up measurements at V3 have shown similar laboratory outcomes in both the studied and control groups throughout the study period (Hb 11.75 ± 1.28 g/dl vs. 11.40 ± 1.10 g/dl, $p = 0.19$ and serum creatinine 0.81 ± 0.21 mg/dl vs. 0.85 ± 0.32 mg/dl, $p = 0.45$).

It is important to note that, in accordance with the hospital protocol for laboratory testing, only Hb and creatinine levels were monitored during the follow-up period (Visit 3). Consequently, while the data provided valuable insights into these specific parameters, the absence of follow-up measurements for other laboratory markers, such as CRP and HSTnI, limits the scope of our post-operative analysis.

TABLE 4 General laboratory tests.

| | | Studied group $N = 40$ | Control group $N = 40$ | $p$ |
|---|---|---|---|---|
| V1 | Hb—g/dl ± SD | 13.66 ± 1.44 | 13.56 ± 1.19 | 0.73 |
| | CRP—mg/dl ± SD | 0.36 ± 0.43 | 0.80 ± 1.60 | 0.10 |
| | HSTnI—pg/ml ± SD | 0.03 ± 0.07 | 0.05 ± 0.12 | 0.57 |
| | Creatinine—mg/dl ± SD | 0.99 ± 0.30 | 0.97 ± 0.30 | 0.83 |
| V2 | Hb—g/dl ± SD | 10.23 ± 1.24 | 10.14 ± 1.00 | 0.71 |
| | CRP—mg/dl ± SD | 6.35 ± 2.69 | 6.43 ± 3.47 | 0.90 |
| | HSTnI—pg/ml ± SD | 0.67 ± 0.69 | 1.22 ± 3.48 | 0,33 |
| | Creatinine—mg/ml ± SD | 1.03 ± 0.35 | 1.10 ± 0.47 | 0.43 |
| V3 | Hb—g/dl ± SD | 11.75 ± 1.28 | 11.40 ± 1.10 | 0.19 |
| | Creatinine—mg/ml ± SD | 0.81 ± 0.21 | 0.85 ± 0.32 | 0.45 |

Hb, hemoglobine; CRP, C reactive protein; HSTnI, high sensitive troponin I.

At the start of the study, a thorough analysis of preoperative biochemical markers was performed, showing that both the studied and control groups had similar plasma levels of SOD-1 and LOX-1 before surgery. This consistency establishes a reliable baseline between the two cohorts, ensuring that any differences in outcomes can be attributed to the interventions rather than to pre-existing variations in these critical markers of oxidative stress and inflammation.

ANOVA analysis of the results of the repeated measures on SOD-1 and LOX-1 levels in patients undergoing coronary artery bypass grafting (CABG) with remote ischemic preconditioning (RIPC) indicated significant differences between the studied and control groups at various time points.

For the studied group, SOD-1 levels significantly increased from V1 (1.38 ± 0.20 pg/ml) to V2 (2.99 ± 0.93 pg/ml, $p < 0.001$) and slightly decreased at V3 (2.20 ± 1.22 pg/ml, $p < 0.01$), yet remained elevated compared to baseline. In contrast, the control group exhibited relatively stable SOD-1 levels, with no significant changes between the visits, as illustrated in Table 5 and Figure 3.

LOX-1 levels showed a different trend, with the studied group experiencing a sharp decrease from V1 (1,205.50 ± 125.18 pg/ml) to V2 (427.52 ± 718.44 pg/ml, $p < 0.001$), followed by a partial rebound at V3 (569.99 ± 607.80 pg/ml, $p < 0.001$). The control group, however, showed a gradual increase in LOX-1 levels over time, from V1 (1,178.58 ± 257.28 pg/ml) to V2 (604.27 ± 403.41 pg/ml) and V3 (749.36 ± 614.75 pg/ml), without reaching statistical significance, as demonstrated in Table 5 and Figure 4.

When comparing the studied and control groups, significant differences emerged at V2 and V3 for both SOD-1 and LOX-1 levels. The studied group exhibited higher SOD-1 levels at both V2 and V3 ($p < 0.01$ and $p < 0.001$, respectively), while LOX-1 levels were significantly lower in the studied group at both V2 and V3 ($p < 0.001$).

# 4 Discussion

The findings of our study suggest that RIPC may modulate oxidative stress and inflammatory responses differently between the groups, contributing to the observed differences in biomarker

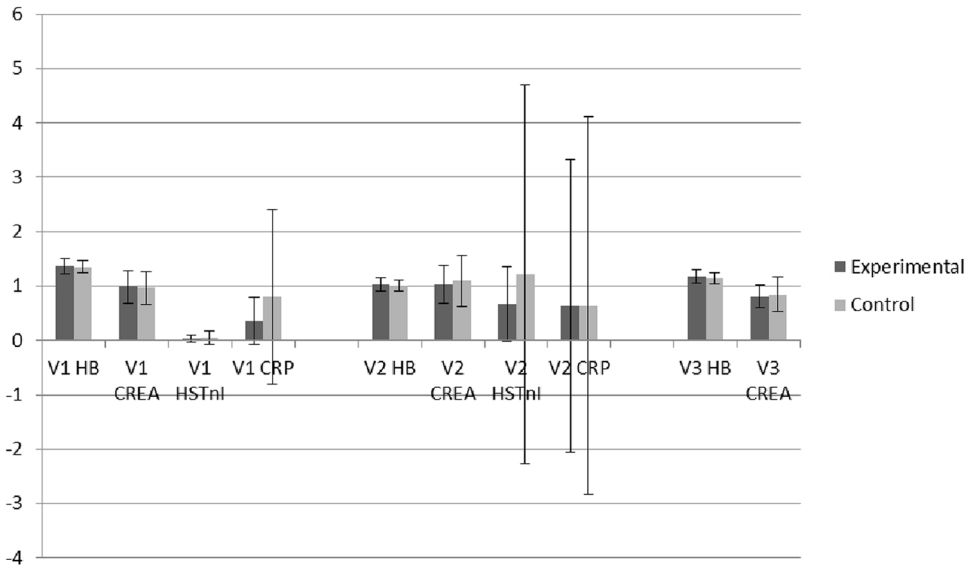


FIGURE 2
Descriptive box plots regarding general laboratory tests activity.

TABLE 5 Results of ANOVA RM for SOD-1 and LOX-1 under RIPC on patients undergoing CABG.

| | SOD 1 | | LOX 1 | |
|---|---|---|---|---|
| | Studied ($n$ = 40) | Control ($n$ = 40) | Studied ($n$ = 40) | Control ($n$ = 40) |
| V1 | 1.38 ± 0.20 | 1.4 ± 0.24 | 1,205.50 ± 125.18 | 1,178.58 ± 257.28 |
| V2 | 2.99 ± 0.93*** | 0.97 ± 0.79 | 427.52 ± 718.44*** | 604.27 ± 403.41 |
| V3 | 2.20 ± 1.22** | 1.41 ± 1.33 | 569.99 ± 607.80*** | 749.36 ± 614.75 |

*post hoc* Comparisons (Holm correction): between (studied vs. control). SOD-1 and LOX-1 values measured in pg/ml.
*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.

levels over time. These data are consistent with previous research assessing the protective role of RIPC.

LOX-1, a receptor for oxidized low-density lipoprotein (oxLDL), plays a crucial role in the pathogenesis of atherosclerosis and ischemia-reperfusion injury. Elevated levels of LOX-1 are associated with endothelial dysfunction, inflammation, and oxidative stress, which are key factors in the progression of coronary artery disease (CAD) and adverse outcomes during CABG (21).

Our findings align with previous studies that have shown RIPC's ability to reduce oxidative stress markers, although few studies have specifically focused on LOX-1. For example, Li et al. reported that RIPC reduced oxidative damage and improved endothelial function in patients undergoing CABG, though LOX-1 was not directly measured (22). The reduction in LOX-1 observed in our study suggests that RIPC may directly downregulate LOX-1 expression or reduce its activation by decreasing the levels of oxLDL, thereby mitigating oxidative stress and endothelial injury during CABG.

SOD-1 is a critical antioxidant enzyme that converts superoxide radicals into hydrogen peroxide, thus protecting cells from oxidative damage (12). The upregulation of SOD-1 observed in our study is consistent with the known mechanisms of RIPC, which is believed to enhance the endogenous antioxidant defense system.

Previous studies have also demonstrated the ability of RIPC to increase antioxidant enzyme levels, although the specific focus on SOD-1 has been limited. However, comparable findings have been reported in studies involving other antioxidant enzymes. For instance, studies by Heusch et al. and Shimizu et al. observed increased levels of antioxidants and reduced oxidative stress in RIPC-treated patients, suggesting that RIPC exerts a broad effect on the antioxidant defense system (9, 23). The significant increase in SOD-1 levels in our study provides further evidence that RIPC can specifically enhance this critical enzyme's activity, potentially contributing to its cardioprotective effects during CABG.

Regarding the comparative analysis of RIPC effects on LOX-1 and SOD-1, one study by Hagiwara et al. explored the impact of RIPC on oxidative stress markers, including LOX-1 and SOD-1, in a rat model of myocardial ischemia. They found that RIPC significantly decreased LOX-1 expression, which is associated with reduced oxidative stress and endothelial dysfunction. Concurrently, RIPC led to a marked increase in SOD-1 levels, suggesting enhanced antioxidant defenses. These findings indicate that RIPC exerts its cardioprotective effects through a dual mechanism: downregulating pro-oxidative pathways involving LOX-1 and upregulating antioxidant enzymes like SOD-1 (24).

Another study by Ding et al. examined the effects of RIPC on LOX-1 and SOD-1 in a diabetic rat model. This study also reported a decrease in LOX-1 levels and an increase in SOD-1 activity following RIPC, supporting the notion that RIPC can mitigate oxidative stress through modulation of these specific biomarkers. The authors suggested that the decrease in LOX-1 might reduce the pro-inflammatory effects of oxidized LDL, while the increase

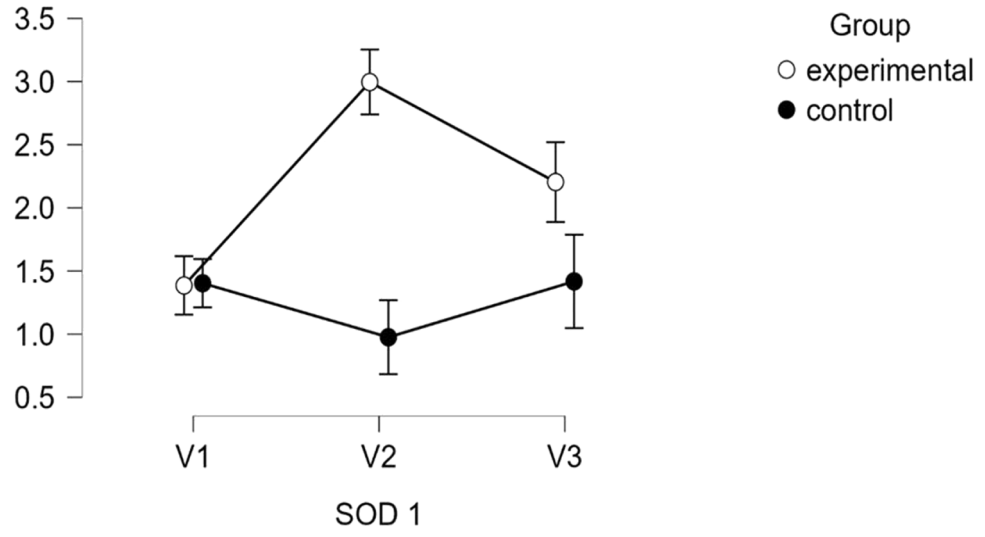


FIGURE 3
Descriptive plots regarding SOD-1 activity.

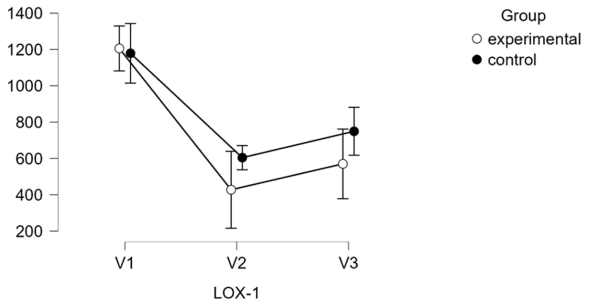


FIGURE 4
Descriptive plots regarding LOX-1 activity.

in SOD-1 could counteract the accumulation of reactive oxygen species (ROS) (25).

The study by Iliodromitis et al. (26) highlights the cardioprotective effects of ischemic preconditioning against myocardial necrosis and apoptosis, both of which are significant concerns during ischemia-reperfusion injury in cardiac surgery. Their research demonstrates that RIPC can mitigate myocardial damage through the activation of various molecular mechanisms, including the reduction of oxidative stress and the inhibition of apoptosis. This is particularly relevant to the current study, which explores the role of antioxidant defense systems, such as SOD-1, and inflamation markers, such as LOX-1, in the context of coronary artery bypass grafting (CABG). The findings from Iliodromitis et al. provide a basis for interpreting how remote ischemic preconditioning (RIPC) might exert similar protective effects in CABG patients by modulating these molecular pathways (26).

While previous research has extensively documented the benefits of RIPC in reducing perioperative myocardial injury and improving clinical outcomes, our findings suggest that the protective mechanisms of RIPC may involve the suppression of pro-oxidative receptors like LOX-1 and the simultaneous upregulation of key antioxidant enzymes like SOD-1.

These findings support the hypothesis that RIPC confers cardioprotection through a multifaceted approach, involving both the attenuation of harmful oxidative processes and the enhancement of protective antioxidant defenses. This dual modulation may be particularly beneficial in the context of CABG, where oxidative stress and inflammation are prevalent and contribute to postoperative complications.

As can be observed in our study also, the methodologies used for determining LOX-1 and SOD-1 in RIPC studies are predominantly centered around ELISA, due to its sensitivity and specificity for detecting these biomarkers in biological fluids. Western blotting and activity assays also play important roles, particularly for tissue-specific studies and functional assessments (27). The selection of methodology depends on the study's focus, whether it is quantifying protein levels or assessing enzymatic activity.

## 4.1 Implications and future directions

Our results contribute to the growing body of evidence that RIPC can modulate oxidative stress and inflammatory pathways, thereby offering potential cardioprotective benefits during surgical interventions like CABG. The results of our study have important clinical implications, suggesting that RIPC could be a valuable strategy to improve outcomes in patients undergoing CABG by targeting specific molecular pathways involved in oxidative stress and inflammation. Future studies should aim to confirm these findings in larger, multicenter trials and explore the mechanistic basis of RIPC's effects on LOX-1 and SOD-1 in greater detail. Additionally, the potential of RIPC as an adjunct

TABLE 6 *Post-hoc* power analysis.

| Power | Without RIPC | With RIPC | Cohen's \|δ\| | α |
|---|---|---|---|---|
| 0.598 | 40 | 40 | 0.500 | 0.050 |
| Power by effect size | | | | |
| True effect size | | Power to detect | | Description |
| $0 < \|\delta\| \leq 0.444$ | | ≤50% | | Likely miss |
| $0.444 < \|\delta\| \leq 0.634$ | | 50%–80% | | Good chance of missing |
| $0.634 < \|\delta\| \leq 0.816$ | | 80%–95% | | Probably detect |
| $\|\delta\| \geq 0.816$ | | ≥95% | | Almost surely detect |

RIPC, remote ischemic preconditioning.

therapy to pharmacological interventions targeting oxidative stress in CABG patients warrants further investigation.

### 4.2 Study limitations

This study has several limitations that should be considered. First, the sample size was relatively small, which may limit the generalizability of the findings, as demonstrated in Table 6.

The post-hoc power analysis presented in Table 6 highlights the limitation of this study stated above. With a sample size of 40 participants per group, the statistical power achieved was 0.598, which falls below the conventional threshold of 0.80, indicating a moderate risk of Type II error. The effect size analysis further underscores this concern, as the study was adequately powered (≥95%) only to detect large effect sizes ($|\delta| \geq 0.816$). For medium effect sizes ($0.634 < |\delta| \leq 0.816$), the power ranged between 80% and 95%, suggesting a reasonable likelihood of detecting differences, though still with some risk of underestimation. However, for smaller effect sizes ($|\delta| \leq 0.634$), the power drops considerably, indicating a substantial chance of missing significant effects. This limitation suggests that the study may not be sufficiently sensitive to detect smaller, yet potentially clinically relevant, differences between groups.

Additionally, the study was conducted in a single center, potentially introducing selection bias. The short-term follow-up period did not allow for the assessment of long-term effects of RIPC on LOX-1 and SOD-1 levels post-CABG. Finally, while we observed significant changes in these biomarkers, the underlying mechanisms of how RIPC modulates LOX-1 and SOD-1 remain unclear, warranting further studies.

## 5 Conclusion

The significant changes in plasma levels of both LOX-1 and SOD-1 observed in this study strongly suggest that remote ischemic preconditioning (RIPC) plays an important role in reducing oxidative stress and enhancing the antioxidative status of patients. This is evidenced by the marked decrease in LOX-1 levels, alongside a corresponding increase in SOD-1 levels, indicating that RIPC may contribute to improved cardioprotection through these mechanisms.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Ethics statement

The studies involving humans were approved by Scientific Research Ethics Committee, Transilvania University of Brașov. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

## Author contributions

C-DL: Formal Analysis, Investigation, Resources, Software, Visualization, Writing – original draft, Writing – review & editing. AB: Investigation, Software, Writing – review & editing, Data curation. DN: Formal Analysis, Investigation, Methodology, Resources, Software, Writing – review & editing, Data curation. DȚ: Conceptualization, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note

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# A nomogram model to predict non-retrieval of short-term retrievable inferior vena cava filters

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**Objective:** To develop and validate a nomogram for predicting non-retrieval of the short-term retrievable inferior vena cava (IVC) filters.
**Methods:** In this study, univariate and multivariate logistic regression analyses were performed to identify predictive factors of short-term retrievable filter (Aegisy or OptEase) non-retrieval, and a nomogram was then established based on these factors. The nomogram was created based on data from a training cohort and validated based on data from a validation cohort. The predictive value of the nomogram was estimated using area under the curve (AUC) and calibration curve analysis (Hosmer-Lemeshow test).
**Results:** A total of 1,321 patients who had undergone placement of short-term retrievable filters (Aegisy or OptEase) were included in the analysis. The overall retrieval rate was 68.7%. Age, proximal and distal deep vein thrombosis (DVT) vs. distal DVT, active cancer, history of long-term immobilization, VTE was detected in the intensive care unit, active/recurrent bleeding, IVC thrombosis, and history of venous thromboembolism were independent predictive risk factors for non-retrieval of filters. Interventional therapy for DVT, acute fracture, and interval of ≥14 days between filter placement and patient discharge were independent protective factors for non-retrieval of filters. The nomogram based on these factors demonstrated good ability to predict the non-retrieval of filters (training cohort AUC = 0.870; validation cohort AUC = 0.813.
**Conclusion:** This nomogram demonstrated strong predictive accuracy and discrimination capability. This model may help clinicians identify patients who are not candidates for short-term retrievable filter placement and help clinicians make timely, individualized decisions in filter choice strategies.

KEYWORDS
inferior vena cava, filter, retrieval, risk factor, OptEase, nomogram

## Introduction

Retrievable inferior vena cava (IVC) filters are devices that provide either temporary or permanent protection against the formation of pulmonary embolism (PE) (1). When a patient's clinical indication for PE protection no longer exists, the retrievable IVC filter can be retrieved to reduce the risk of potential long-term complications (2). There are two types of retrievable IVC filters commonly used in clinical practice, one is the "spindle" filter with retrieval window of 14 days, another is "umbrella" filter with no

clear retrieval window (usually weeks to months) (3). The "spindle" filter is often not retrieved because the risk of PE is not eliminated at the end of retrieval window (Figure 1) (4). Long-term retention of the short-term filters may lead to complications (2). How to reasonably select different types of filters according to the situation of patients, to avoid the "spindle" filter non-retrieved due to the wrong choice strategy, is a clinical problem to be solved. The Aegisy (Lifetech Scientific, Shenzhen, China) and OptEase (Cordis, Santa Clara, California, USA) are the most commonly used short-term filters in China. They are similar in appearance and clinical characteristics (Figure 2).

The aim of this study was to determine the factors associated with non-retrieval of these short-term filters (Aegisy and OptEase) and to establish and validate a nomogram for predicting the probability of non-retrivability, so as to better guide physicians to choose the appropriate type of filter.

# Materials and methods

## Study design

The study population consisted of a training cohort and a validation cohort. The training cohort included patients with short-term filters (Aegisy or OptEase) filters placed at the affiliated changzhou second people's hospital of nanjing medical university (Hospital A) from January 2016 to May 2022. The validation cohort included patients with short-term filters (Aegisy or OptEase) filters placed at the first affiliated hospital of soochow university (Hospital B) from January 2016 to May 2022. All filter placements and venous thromboembolism (VTE) treatment were based on ESC guidelines for the diagnosis and management of acute PE and expert consensus guidelines from the Chinese College of Interventionalists (CCI) (4, 6, 7). The retention time of Aegisy and OptEase filters is normally ≤14 days.

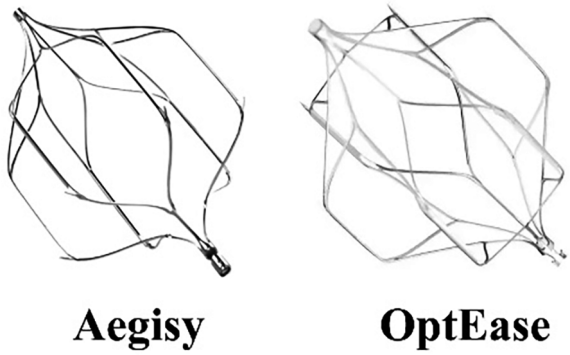


FIGURE 2
Images of Aegisy and OptEase filters. The short-term filters with a spindle-shaped appearance improve stability but also increase the contact area between the struts and the IVC wall, which can easily cause intimal hyperplasia to encase the struts.

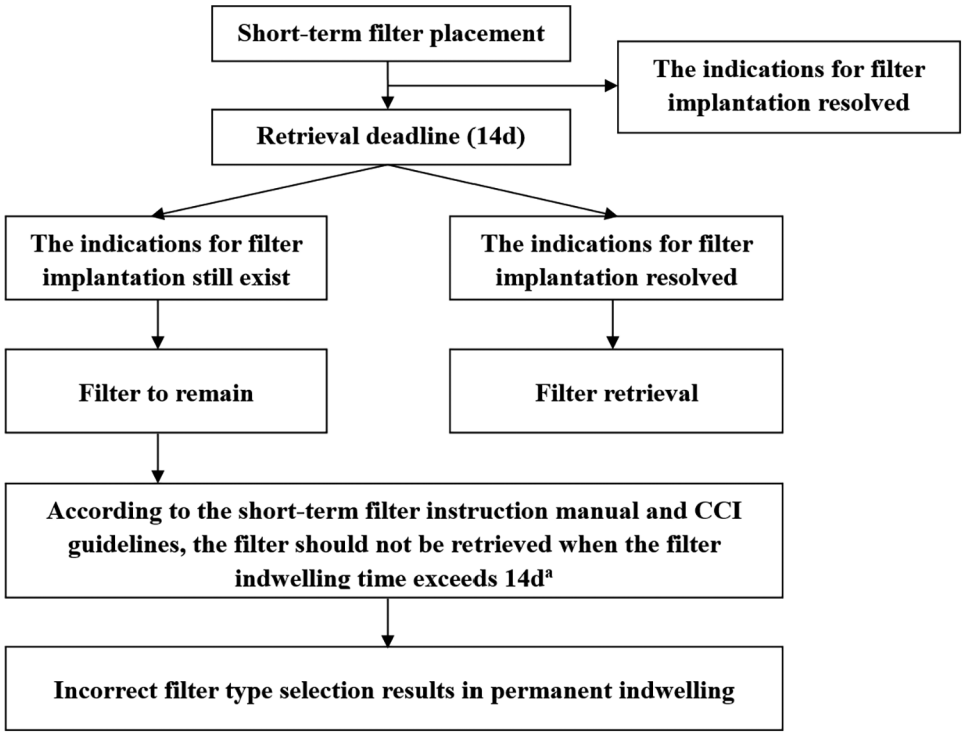


FIGURE 1
Short-term filter use patterns. CCI, Chinese College of Interventionalists. [a]Vena cava endothelial hyperplasia and adhesion of the filters to the wall of the vena cava occurred when the short-term filter indwelling time exceeds 2 weeks (5).

The training and validation cohorts were subdivided into filter retrieval and non-retrieval groups. The nomogram was established using the training cohort and was validated using the validation cohort. All data were anonymized, and personal identifiers were completely deleted.

## Inclusion and exclusion criteria

Cases were included in the analysis if (1) the type of IVC filter placed was Aegisy or OptEase; (2) the filter placement was intended to be temporary. Cases were excluded from the final analysis if (1) the patients <18 years old; (2) patients with short life expectancy (not being requested to make a retrieval attempt); (3) the patient had died before filter retrieval attempt; (4) the patient had been lost to follow-up.

## Data collection

A list of potential predictors of filter non-retrieval was compiled based on clinical judgment and a search of the relevant literature (8–11). These predictors included (1) clinical characteristics of patients (sex, age, indication for filter placement, history of long-term immobilization, and department in which VTE was detected); (2) the occurrence of VTE events [clinical classification of deep vein thrombosis (DVT) or treatment of VTE]; and (3) the presence of concomitant conditions, including acute fracture (occurred in the previous 2 weeks), active cancer, acute cerebral hemorrhage/infarction, active/recent bleeding, iliac vein compression syndrome (IVCS), or IVC thrombosis.

## Statistical analysis

SPSS version 26.0 (IBM Corp, Armonk, NY, USA) was used for data analysis. Measurement data were expressed as M ($P_{25}$, $P_{75}$), with rank-sum tests used to compare groups. Count data were expressed as frequency (percentage), with differences between groups analyzed using chi-square tests or Fisher's exact probability method. $P$ values less than 0.05 were considered statistically significant.

## Construction of the nomogram

Univariate and multivariate logistic regression analyses were used to determine the independent predictors of non-retrieval for short-term filters filters in the training cohort. Variables significantly related to the probability of non-retrieval in the univariate logistic regression analysis ($P < 0.05$) were subsequently included in the multivariate regression analysis. The nomogram was then constructed using *R* software (version 4.2.0) to visually score the individual probabilities for short-term filters filters non-retrieval.

## Performance of the nomogram

Receiver operating characteristic (ROC) curves and calibration curves were constructed to estimate the value of the nomogram in the training and validation cohorts. The discrimination performance of the nomogram was assessed using area under the curve (AUC). Calibration of the nomogram was evaluated using a calibration curve and a Hosmer-Lemeshow test [nonsignificance ($P > 0.05$) of the Hosmer-Lemeshow test indicates good agreement].

## Ethics approval

The study was conducted following the Declaration of Helsinki (as revised in 2013), and approved by the institutional review boards with waivers of informed consent. All data collection and analysis processes were performed in accordance with the institutional review board regulations.

# Results

## Patients

During the study period, Short-term filters were placed in 1,608 patients. Of these patients, 287 were excluded from the analysis. The final study population therefore included 1,321 patients (826 patients in the training cohort, 495 patients in the validation cohort) (Figure 3). The overall filter retrieval rate was 68.7% (63.2% in the training cohort, 78.0% in the validation cohort). The reasons for the short-term filters filters non-retrieval were as follows: (1) propagation/progression of VTE despite appropriate anticoagulation (65.9%); (2) inability to maintain adequate anticoagulation, or complication of anticoagulation (21.3%); (3) contraindications to anticoagulation still exists (12.8%). Further information about filter placement and retrieval is shown in Table 1.

## Independent predictors

Univariate analysis of the training cohort identified the following significant predictors of filter non-retrieval: patient age, DVT classification, interventional therapy for DVT, acute fracture, active cancer, IVCS, acute cerebral hemorrhage/infarction, history of long-term immobilization, DVT in ICU patient, active/recent bleeding, IVC thrombosis, history of VTE, and interval of ≥14 d between filter placement and patient discharge. Similar results were seen in the validation cohort (Table 2).

In multivariate logistic regression analysis, age [odds ratio [OR] = 1.071; 95% confidence interval [CI]: 1.052–1.090], both proximal and distal DVT vs. distal DVT (OR = 1.763; 95% CI: 1.081–2.874), active cancer (OR = 12.112; 95% CI: 7.169–20.461), history of long-term immobilization (OR = 35.962; 95% CI: 12.503–103.441), DVT in ICU patient (OR = 3.807; 95% CI: 1.642–8.825), active/recent bleeding (OR = 4.879; 95% CI:

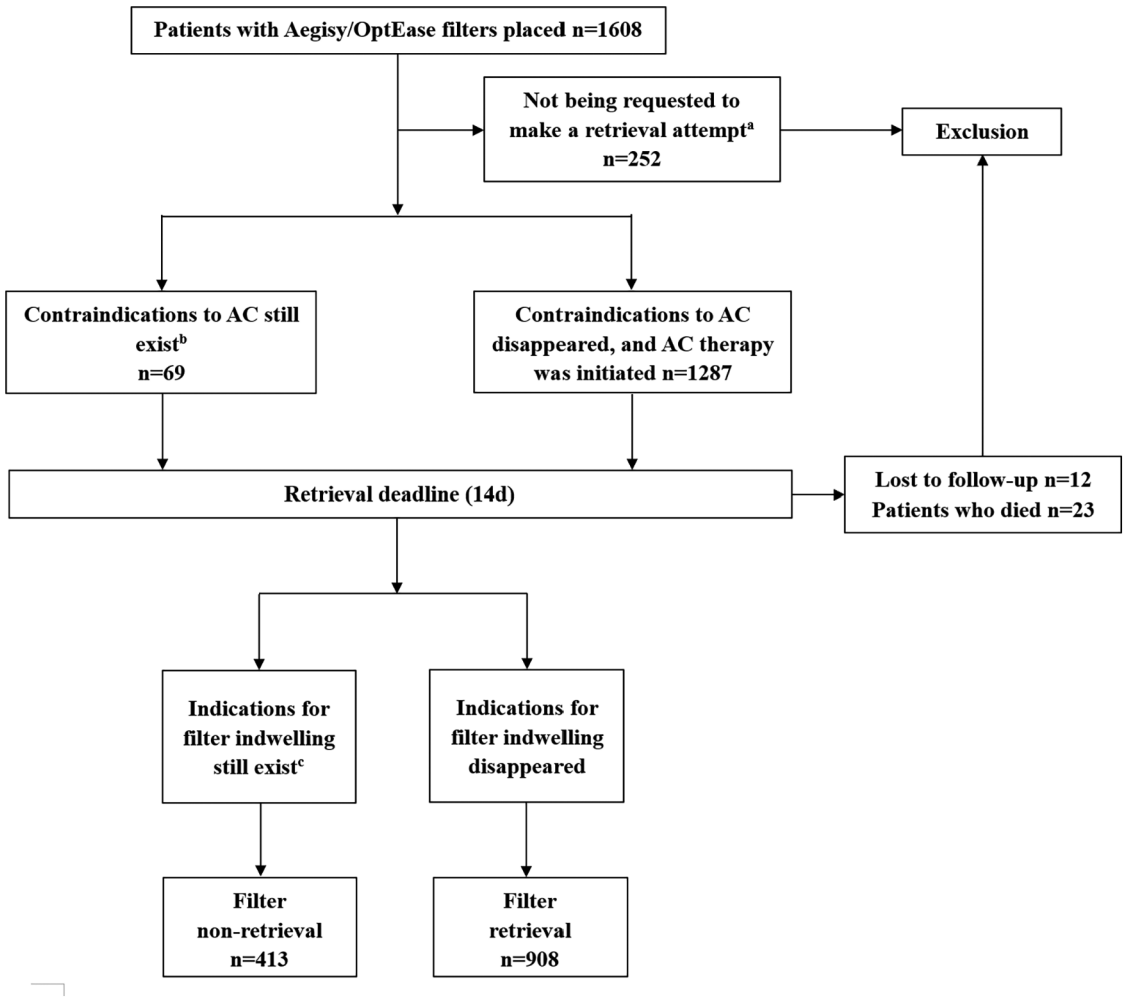


FIGURE 3
Patient selection flowchart. AC, anticoagulation. [a]Patients with short life expectancy (discharge to hospice). [b]Active/recent bleeding was not corrected. [c]Propagation/progression VTE despite appropriate AC, inability to maintain adequate anticoagulation or complication of anticoagulation needs to be stopped.

TABLE 1 Characteristics of filter placement and retrieval among study patients (*N* = 1,321).

| Characteristic | No. of patients (%) |
|---|---|
| Indication for filter placement | |
| VTE with contraindication to AC | 586 (44.4) |
| VTE with peri-operative needs to stop AC | 459 (34.7) |
| Progression VTE despite appropriate AC | 204 (15.4) |
| Recurrent VTE despite appropriate AC[a] | 72 (5.5) |
| Type of filter | |
| Aegisy | 1,169 (88.5) |
| OptEase | 152 (11.5) |
| Filters retrieved[b] | 908 (68.7) |
| Reason for non-retrieval of short-term filter | |
| Propagation/progression of VTE despite appropriate AC | 272 (65.9) |
| Inability to maintain adequate AC, or complication of AC | 88 (21.3) |
| Contraindications to AC still exists | 53 (12.8) |

AC, anticoagulation; VTE, venous thromboembolism.
[a]During a single hospitalization, VTE recurred after treatment.
[b]All filters were retrieved within the time specified by the manufacturer (14 d), and there was no failure of retrieved due to complications; Patients with thrombosis in the filter, the filter was all retrieved safely after thrombus aspiration.

2.202–10.812), IVC thrombosis (OR = 13.116; 95% CI: 3.215–53.507), and history of VTE (OR = 12.534; 95% CI: 2.013–78.030) were identified as independent risk factors for non-retrieval of short-term filters filters. Interventional therapy for DVT (OR = 0.353; 95% CI: 0.202–0.619), acute fracture (OR = 0.461; 95% CI: 0.280–0.758), and interval of ≥14 d between filter placement and patient discharge (OR = 0.435; 95% CI: 0.286–0.660) were independent protective factors for non-retrieval of short-term filters filters (Table 3).

## Development and validation of a predictive nomogram

We used these 11 predictors of non-retrieval in a binary logistic regression analysis and transformed the results into a nomogram that could be used to predict the probabilities of filter non-retrieval (Figure 4). With this nomogram, the age of the patient was positioned on the corresponding variable axis;

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TABLE 2 Univariate logistic regression analysis for predictors of filter non-retrieval.

| Variable | Training cohort, *n* (%) | | | Validation cohort, *n* (%) | | |
|---|---|---|---|---|---|---|
| | Retrieval group (*n* = 522) | Non-retrieval group (*n* = 304) | *P* value | Retrieval group (*n* = 386) | Non-retrieval group (*n* = 109) | *P* value |
| Sex | | | 0.642 | | | 0.789 |
| Male | 256 (49.0) | 144 (47.4) | | 172 (44.6) | 47 (43.1) | |
| Female | 266 (51.0) | 160 (52.6) | | 214 (55.4) | 62 (56.9) | |
| Age, y | 65 (56, 72) | 73 (65, 80) | <0.001 | 63 (52, 72) | 70 (62, 80) | <0.001 |
| DVT clinical classification[a] | | | 0.017 | | | 0.017 |
| Proximal DVT | 112 (21.5) | 58 (19.1) | | 58 (15.0) | 22 (20.2) | |
| Distal DVT | 153 (29.3) | 73 (24.0) | | 72 (18.7) | 10 (9.2) | |
| Both proximal and distal DVT | 158 (30.3) | 125 (41.1) | | 256 (66.3) | 76 (69.7) | |
| PE only[b] | 99 (18.9) | 48 (15.8) | | 0 (0) | 1 (0.9) | |
| Interventional therapy for DVT | | | <0.001 | | | <0.001 |
| Yes | 123 (23.6) | 30 (9.9) | | 216 (56.0) | 17 (15.6) | |
| No | 399 (76.4) | 274 (90.1) | | 170 (44.0) | 92 (84.4) | |
| PE | | | 0.202 | | | 0.666 |
| Yes | 148 (28.4) | 99 (32.6) | | 179 (46.4) | 48 (44.0) | |
| No | 374 (71.6) | 205 (67.4) | | 207 (53.6) | 61 (56.0) | |
| Interventional therapy for PE | | | 0.187 | | | 0.154 |
| Yes | 49 (33.1) | 25 (25.3) | | 80 (44.7) | 27 (56.2) | |
| No | 99 (66.9) | 74 (74.7) | | 99 (55.3) | 21 (43.8) | |
| Acute fracture[c] | | | <0.001 | | | 0.026 |
| Yes | 177 (33.9) | 50 (16.4) | | 116 (30.1) | 21 (19.3) | |
| No | 345 (66.1) | 254 (83.6) | | 270 (69.9) | 88 (80.7) | |
| Fracture site | | | 0.090 | | | 0.628 |
| Centrum | 4 (2.3) | 5 (10.0) | | 19 (16.4) | 4 (19.0) | |
| Femur | 90 (50.8) | 19 (38.0) | | 50 (43.1) | 11 (52.4) | |
| Long bones/joints of the extremities other than femur | 35 (19.8) | 10 (20.0) | | 18 (15.5) | 1 (4.8) | |
| Multiple fractures | 48 (27.1) | 16 (32.0) | | 29 (25.0) | 5 (23.8) | |
| Active cancer | | | <0.001 | | | <0.001 |
| Yes | 30 (5.7) | 96 (31.6) | | 68 (17.6) | 46 (42.2) | |
| No | 492 (94.3) | 208 (68.4) | | 318 (82.4) | 63 (57.8) | |
| IVCS[d] | | | <0.001 | | | <0.001 |
| Yes | 72 (13.8) | 16 (5.3) | | 140 (36.3) | 20 (18.3) | |
| No | 450 (86.2) | 288 (94.7) | | 246 (63.7) | 89 (81.7) | |
| Acute cerebral hemorrhage/ infarction | | | 0.001 | | | <0.001 |
| Yes | 10 (1.9) | 20 (6.6) | | 25 (6.5) | 22 (20.2) | |
| No | 512 (98.1) | 284 (93.4) | | 361 (93.5) | 87 (79.8) | |
| History of long-term immobilization[e] | | | <0.001 | | | 0.036 |
| Yes | 5 (1.0) | 46 (15.1) | | 148 (38.3) | 54 (49.5) | |
| No | 517 (99.0) | 258 (84.9) | | 238 (61.7) | 55 (50.5) | |
| DVT in ICU patient | | | <0.001 | | | <0.001 |
| Yes | 32 (6.1) | 42 (13.8) | | 33 (8.5) | 24 (22.0) | |
| No | 490 (93.9) | 262 (86.2) | | 353 (91.5) | 85 (78.0) | |
| Active/recent bleeding[f] | | | <0.001 | | | 0.019 |
| Yes | 36 (6.9) | 45 (14.8) | | 26 (6.7) | 15 (13.8) | |
| No | 486 (93.1) | 259 (85.2) | | 360 (93.3) | 94 (86.2) | |
| IVC thrombosis[g] | | | 0.031 | | | 0.019 |
| Yes | 6 (1.1) | 10 (3.3) | | 10 (2.6) | 8 (7.3) | |
| No | 516 (98.9) | 294 (96.7) | | 376 (97.4) | 101 (92.7) | |
| History of VTE[h] | | | 0.027 | | | <0.001 |
| Yes | 2 (0.4) | 7 (2.3) | | 11 (2.8) | 16 (14.7) | |
| No | 520 (99.6) | 297 (97.7) | | 375 (97.2) | 93 (85.3) | |

(*Continued*)

TABLE 2 Continued

| Variable | Training cohort, *n* (%) | | | Validation cohort, *n* (%) | | |
|---|---|---|---|---|---|---|
| | Retrieval group (*n* = 522) | Non-retrieval group (*n* = 304) | *P* value | Retrieval group (*n* = 386) | Non-retrieval group (*n* = 109) | *P* value |
| Interval of ≥14 d between filter placement and patient discharge[i] | | | <0.001 | | | 0.047 |
| Yes | 257 (49.2) | 105 (34.5) | | 119 (30.8) | 23 (21.1) | |
| No | 265 (50.8) | 199 (65.5) | | 267 (69.2) | 86 (78.9) | |

DVT, deep vein thrombosis; ICU, intensive care unit; IVC, inferior vena cava; IVCS, iliac vein compression syndrome; PE, pulmonary embolism; VTE, venous thromboembolism.
[a]According to the location: Distal DVT refers to distal (or calf) DVT in the legs when it is found below the knee; Proximal DVT means a proximal (or iliofemoral) DVT in the legs above the knee.
[b]PE only and contraindicated with anticoagulation.
[c]Occurred in the previous 2 weeks.
[d]Diagnosed via angiography before filter placement.
[e]Long-term bed rest for more than 3 months.
[f]VTE was detected while the patient is experiencing active/recurrent bleeding.
[g]Thrombosis extending from the iliac vein to the inferior renal segment of the IVC, thrombus aspiration therapy was performed after filter placement over the IVC thrombus.
[h]Occurring 3 or more months prior to filter placement.
[i]14 days was the end of filter retrieval window, the interval between filter placement and patient discharge ≥14 days, which meant that the patient completed filter placement and retrieval within one hospitalization.

TABLE 3 Multivariable logistic regression analysis for predictors of filter non-retrieval.

| Variable | OR (95% CI) | *P* value |
|---|---|---|
| Age, y | 1.071 (1.052–1.090) | <0.001 |
| DVT classification | | 0.020 |
| Proximal DVT | 1.148 (0.663–1.986) | 0.623 |
| Both proximal and distal DVT | 1.763 (1.081–2.874) | 0.023 |
| Only PE | 0.799 (0.453–1.410) | 0.439 |
| Interventional therapy for DVT (yes/no) | 0.353 (0.202–0.619) | <0.001 |
| Acute fracture (yes/no) | 0.461 (0.280–0.758) | 0.002 |
| Active cancer (yes/no) | 12.112 (7.169–20.461) | <0.001 |
| History of long-term immobilization (yes/no) | 35.962 (12.503–103.441) | <0.001 |
| DVT in ICU patient (yes/no) | 3.807 (1.642–8.825) | 0.002 |
| Active/recent bleeding (yes/no) | 4.879 (2.202–10.812) | <0.001 |
| IVC thrombosis (yes/no) | 13.116 (3.215–53.507) | <0.001 |
| History of VTE (yes/no) | 12.534 (2.013–78.030) | 0.007 |
| Interval of ≥14 d between filter placement and patient discharge (yes/no) | 0.435 (0.286–0.660) | <0.001 |

CI, confidence interval; DVT, deep vein thrombosis; ICU, intensive care unit; IVC, inferior vena cava; OR, odds ratio; PE, pulmonary embolism; VTE, venous thromboembolism.

next, a vertical line was drawn to the "Points" axis to obtain the corresponding score (e.g., when age = 60, the corresponding score was 50). These steps were then repeated to obtain the scores for each variable, and all scores were summed to obtain the total score. This total score was identified on the "Total points" axis, and a vertical line was drawn to the "probabilities of non-retrieval" axis to determine the risk probabilities of non-retrieval.

The nomogram demonstrated a good ability to predict the non-retrieval of short-term filters. The AUCs were 0.870 (95% CI: 0.845–0.892) for the training cohort and 0.813 (95% CI: 0.775–0.846) for the validation cohort (Figure 5). The calibration curves of the nomogram showed good agreement between prediction and observation. The Hosmer-Lemeshow test was not significant in the training cohort ($P = 0.052$) or in the validation cohort ($P = 0.070$), which indicated a high reliability of the nomogram's predictive ability (Figure 6).

## Discussion

Short-term IVC filters are widely used in clinical practice, as they provide effective protection against PE and can be retrieval once they are no longer needed, and also has the advantage of not prone to tilt (12). In some cases, by the time the retrieval window for short-term filters arrives, the risk of PE and the contraindication to anticoagulation may still be present. These filters are often not retrieved because of the wrong strategy in choice of filter type, and long-term retention of the filters may lead to complications (13).

Previous research has focused on prediction model of non-retrieval filters to guide clinicians to decide whether to use temporary or permanent filters (14–18). This study was designed to identify risk factors for indications beyond the retrieval time window to guide clinicians to decide whether to use short-term or long-term filters. In this study, we found that short-term filters were retrieved in 68.7% of cases. The predictors of non-

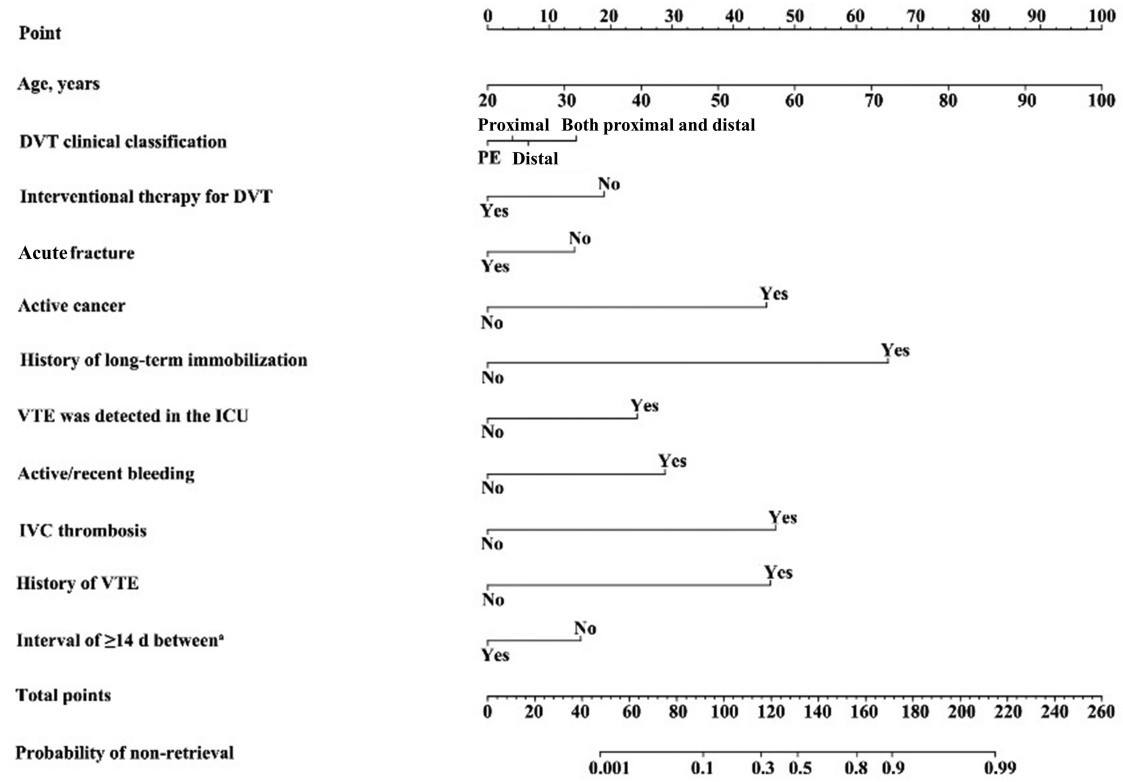


FIGURE 4
Predictive nomogram for risk of non-retrieval of Aegisy and OptEase filters. The points corresponding to each prediction variable were obtained. The sum of the points was then calculated as the total score, and the predicted risk corresponding to the total score was defined as the probability of filter non-retrieval. PE, pulmonary embolism only. [a]Interval of ≥14 d between filter placement and patient discharge.

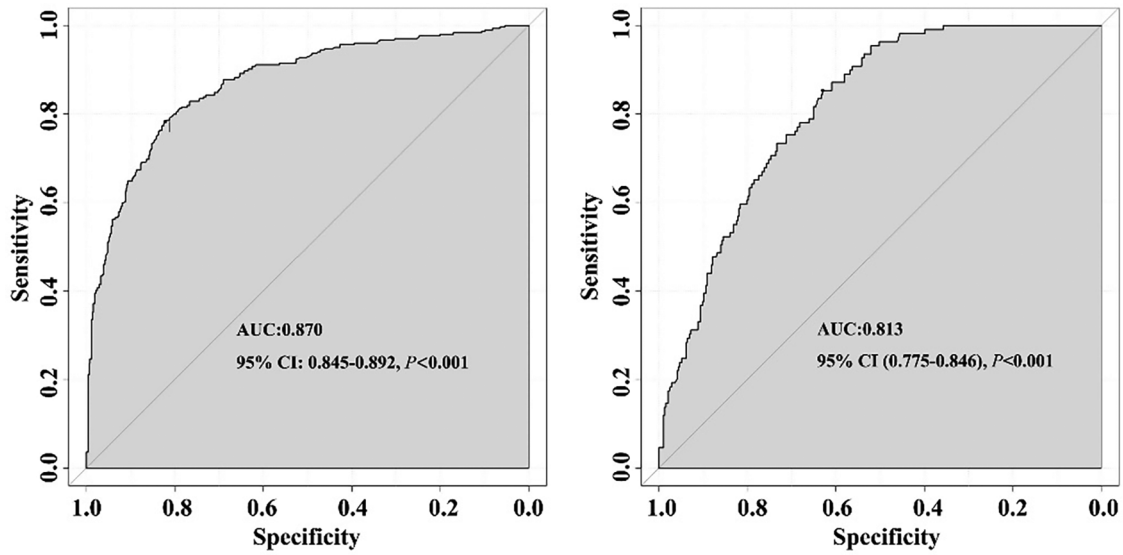


FIGURE 5
Receiver operating characteristic curves for validating the discrimination power of the nomogram prediction model. Training group. Validation group. AUC, area under the curve; CI, confidence interval.

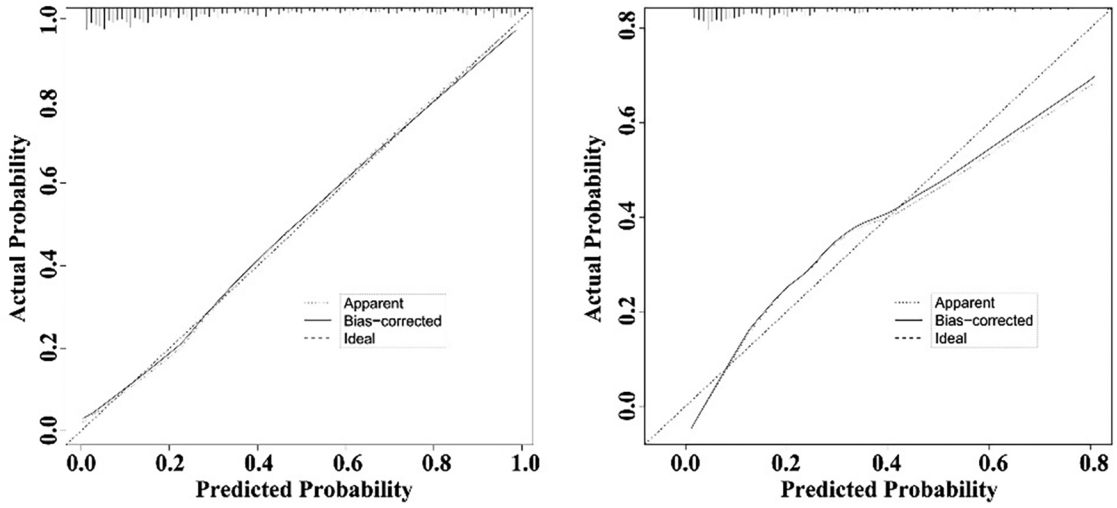


FIGURE 6
Calibration of the nomogram for the probability of non-retrieval of Aegisy and OptEase filters in training cohort and validation cohort. The Hosmer-Lemeshow test was not significant in the training cohort ($P = 0.052$) or in the validation cohort ($P = 0.070$).

retrieval included age, DVT clinical classification, interventional therapy for DVT, acute fracture, active cancer, history of long-term immobilization, DVT in ICU patient, active/recent bleeding, IVC thrombosis, history of VTE, and interval of ≥14 days between filter placement and patient discharge. A nomogram we constructed based on these factors was found to offer a strong ability to predict non-retrieval of these filters.

Advanced age has previously been shown to increase the risk of bleeding during anticoagulation (14). With advancing age, venous valves degenerate, leading to valve incompetence, venous reflux, and increased venous pressure, all of which predispose patients to thrombus formation. Valve damage not only increases the risk of initial DVT but is also associated with post-thrombotic syndrome, resulting in persistent venous insufficiency and recurrent DVT (19–21). Moreover, due to the pharmacokinetic and pharmacodynamic changes in elderly patients, adjusting anticoagulant doses is complex. Even at standard therapeutic doses, elderly patients may experience bleeding complications, necessitating lower doses or more frequent monitoring of coagulation parameters, which can affect the effectiveness of anticoagulant therapy (21). Therefore, elderly patients with DVT present challenges in treatment and have a higher risk of recurrence. In our study, 191 patients were over 80 years old. Of these, 113 (59.2%) demonstrated acute DVT on re-examination, which resulted in the inability to retrieve the filter in a timely manner.

History of long-term immobilization is another known risk factor for VTE. In patients immobilized over a long period, blood flow is slowed and thrombosis is promoted (22). In this study, 253 patients had experienced long-term immobilization when VTE was detected; of these patients, 100 (39.5%) had progression of DVT during treatment, resulting in filters that could not be retrieved.

In this study, we found that fracture was an independent protective factor of the risk of filter non-retrieval. Although fracture itself is a risk factor for DVT, we found that in 1,321 patients, the filter retrieval rate in patients with fracture was higher than in patients without fracture (80.5% vs. 64.3%; $P < 0.05$). In our study, most patients with fractures were short-term contraindicated to anticoagulation or short-term discontinued anticoagulation due to surgery, so patients with fractures could be treated with timely anticoagulation after a brief observation. In addition, previous studies have shown that DVT in patients with fractures is usually found early thanks to standardization of early screening (23, 24). The key to treating DVT is early management (22). For patients with DVT due to fracture, most of the indications for filter placement have usually been eliminated within the deadline of filter retrieval, leading to high retrieval rates.

Active cancer is another risk factor for VTE (25). In addition, the risk of bleeding is substantially increased in patients with cancer because of the risk of thrombocytopenia after treatment, which has led to concerns regarding the use of anticoagulant drugs in these patients (26–28). In the current study, 240 (18.2%) patients had active cancer, filter placement was performed because of poor general condition, insufficient anticoagulation, and progression or recurrence of VTE. 183 (76.3%) of these were found to have DVT progression or large residual DVT at the time of proposed filter retrieval. Therefore, short-term filters should be used with caution in patients with active cancer, and clinicians must choose the type of filter based on the overall condition of the patient, life expectancy, and medical situation.

VTE detected in the ICU are usually in critically ill, and most of these patients have indwelling arteriovenous catheters, which increases the risk of VTE (29). In this study, DVT in ICU patient in 66 patients (50.4%) with contraindications to anticoagulation. They could not undergo filter retrieval because of an inability to maintain adequate anticoagulation and the progression of DVT.

History of VTE is another factor that must be considered in patients receiving short-term filters. In this study, 36 patients had a history of VTE and 23 patients (63.9%) were deemed non-retrievable due to progression of DVT. In such patients, it may be related to the presence of risk factors for thrombosis and an unreasonable anticoagulation regimen; the patient's compliance with this anticoagulation program may be poor. For patients with recurrent VTE, clinicians should therefore not only screen for refractory thromboembolism but should also adjust the anticoagulant treatment plan and strengthen follow-up. At the same time, short-term filters should be avoided as much as possible.

Patients with both proximal and distal DVT and IVC thrombosis are also at greater risk of DVT formation and thrombosis (22). In these patients, because of the large extent of the thrombosis, reflux can lead to decompensation, resulting in blood stasis, which can in turn aggravate the progression of DVT (22). Therefore, some of these patients who undergo short-term filters placement will continue to have a risk of PE that is not eliminated before the filter retrieval deadline, leading to indwelling of the filters.

Research has shown that interventional therapy for DVT can improve the rate of complete recanalization of the lumen, prevent venous valve adhesion, and reduce the incidence of valvular insufficiency and thrombosis recurrence (7). Therefore, DVT is cleared faster when interventional therapy is used. In this study, the use of interventional therapy was found to be a protective factor for non-retrieval of filters. Similarly, an interval of ≥14 days between filter placement and patient discharge was demonstrated to be a protective factor, perhaps because VTE in these patients was better prevented and treated in our department. Additionally, these patients were less likely to be lost to follow-up, since they did not need to readmission after discharge for retrieval after filter placement.

Overall, these results confirm the importance of filter choice when treating patients for VTE. The clinician can use this normogram to identify patients who are not candidates for short-term retrievable filter placement and conduct an individualized assessment of the patient receiving the retrievable filters placement to guide the clinician in selecting the appropriate type of filter and avoid associated complications caused by incorrect choice leading to long time indwelling of the filter. Our findings also suggest that clinicians should improve the supervision system of anticoagulation therapy, and that interventional thrombectomy should be performed when necessary to prevent the non-retrieval of filters because of the presence of residual.

This study had several limitations. First, although many risk factors can affect filter retrieval, our analysis included only the most important variables that can be readily assessed in clinical practice. Second, our model was only validated in the same region, and the performance of the model in regions with different management strategies is unknown. Finally, there was a difference in the retrieval rate between the training group and the validation group. The placement and retrieval of short-term filters in the two groups followed the same guidelines, and the difference in retrieval rate may be due to the difference in disease composition between the two groups.

In conclusion, this study demonstrated that age, DVT clinical classification, active cancer, history of long-term immobilization, VTE was detected in the ICU, active/recent bleeding, IVC thrombosis, history of VTE, interventional therapy for DVT, acute fracture, and interval of ≥14 days between filter placement and patient discharge were predictors of non-retrieval for short-term filters. The nomogram constructed in this study can provide clinicians with information to guide clinical decision-making and alter their strategy in choice of filter type to maximize the benefits for patients.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Ethics statement

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

## Author contributions

LQ: Data curation, Formal Analysis, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. XG: Writing – original draft, Conceptualization, Methodology. CN: Writing – original draft, Investigation, Supervision. KW: Methodology, Writing – original draft, Conceptualization. TX: Writing – original draft, Writing – review & editing, Data curation. ZJ: Data curation, Methodology, Writing – original draft, Writing – review & editing. YW: Data curation, Methodology, Supervision, Formal Analysis, Writing – original draft.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



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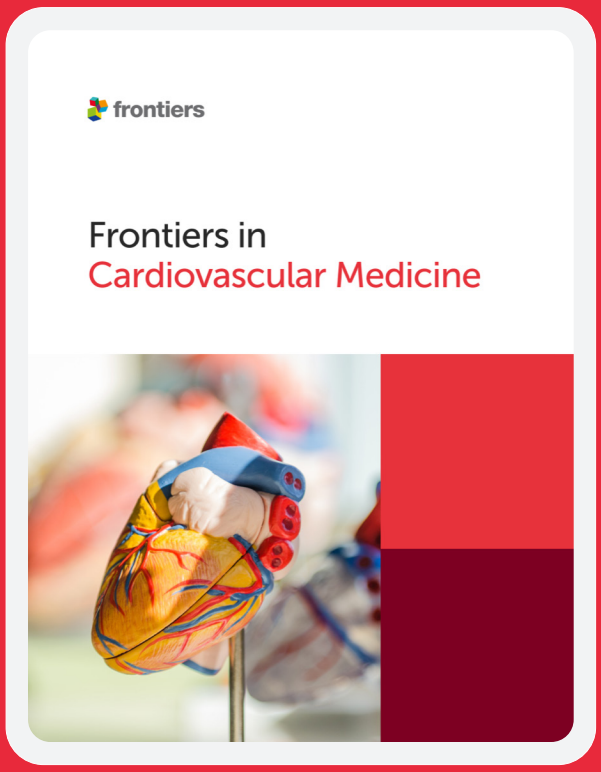


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