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# Progress in Serum Metabolomics Research on Venous Thromboembolic Diseases

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**Abstract:** ***Venous thromboembolic disease (VTE), comprising deep vein thrombosis (DVT) and pulmonary embolism (PE), exhibits rising incidence and mortality rates due to the lack of early, sensitive, and specific diagnostic biomarkers. Serum metabolomics, a branch of metabolomics, focuses on studying the dynamic changes of all small-molecule metabolites in serum. In recent years, numerous researchers have identified serum differential metabolites associated with VTE development through serum metabolomics studies in VTE patients. By investigating their metabolic pathways, these studies suggest that the pathogenesis may be linked to abnormalities in lipid metabolism, amino acid metabolism, energy metabolism, and adenosine metabolism. Serum metabolomics has revealed key metabolic abnormalities in VTE progression, offering new perspectives for early diagnosis, mechanism elucidation, and targeted therapy. However, its clinical application still faces numerous challenges.***

**Keywords:** Deep Vein Thrombosis, Serum Metabolomics, Pathogenesis**.**

## 1. Introduction

Deep vein thrombosis (DVT) is a common vascular disorder triggered by blood stasis, vascular endothelial injury, and hypercoagulable states. DVT fragments can detach and cause pulmonary embolism (PE); collectively, DVT and PE are termed venous thromboembolism (VTE) [1]. The absence of early, sensitive, and specific diagnostic markers for deep vein thrombosis significantly increases the risk of PE. Metabolomics refers to the comprehensive and systematic analysis of all low-molecular-weight compounds that are intermediate or final products of metabolism, resulting from biochemical and physiological processes within the body and exhibiting differential abundance in biological fluids, cells, and tissues [2]. Serum metabolomics is a branch of this field. Numerous research trials have employed metabolomic analysis to measure DVT metabolites, thereby revealing a glimpse into the microscopic pathogenesis of DVT and providing references for early, sensitive, and specific diagnostic markers for DVT.

## 2. Serum Metabolomics

Metabolomics, an emerging omics technology following genomics, transcriptomics, and proteomics, encompasses all low-molecular-weight (MW<1000) chemical products within a given organism or cell during specific physiological periods [3,4]. Its primary analytical techniques fall into two categories: mass spectrometry and nuclear magnetic resonance (NMR), encompassing liquid chromatography (LC)-MS, gas chromatography (GC)-MS, ultra-high-performance liquid chromatography (UHPLC)-MS, flow injection analysis (FIA)-MS, mass spectrometry imaging (MSI), and capillary electrophoresis (CE), among others [5]. Serum metabolomics employs analytical techniques like (LC)-MS and (GC)-MS to investigate the types, concentrations, and dynamic changes of small-molecule metabolites in serum. Through high-throughput analysis, it reveals metabolic signatures in physiological or pathological states, enabling the identification of potential biomarkers. With technological advancements, metabolomics has been applied to the early diagnosis of various diseases and the investigation of their pathophysiology and pathogenesis. Metabolomics-based research on VTE may identify potential early diagnostic metabolites, elucidate the pathogenesis of VTE, and provide insights for its early diagnosis and the prevention of PE. This review explores advances in serum metabolomics research related to VTE by focusing on one branch of metabolomics, offering new perspectives for clinical applications of VTE.

## 3. Advances in Serum Metabolomics Research Applied to VTE

Through serum metabolomics studies in animal models and/or patients with VTE, a series of potential biomarkers with good sensitivity and specificity for early VTE diagnosis have been identified. These include glycolysis-related metabolites (pyruvate, lactate), tricarboxylic acid cycle intermediates (citrate, malate), amino acids (valine, phenylalanine, alanine, tryptophan, glutamine), lipids (sphingomyelin, phosphatidylcholine, acylcarnitines, triglycerides, ceramides), acetoacetic acid, carnitines, adenosine, and kynurenine. This paper attempts to analyze the pathogenesis based on serum metabolites and identify potential early diagnostic biomarkers for clinical application.

## 4. Serum Metabolomics Pathogenesis of VTE

It is well known that German scholar Virchow summarized the primary pathogenesis of DVT from a macro perspective: venous wall injury, slow blood flow, and hypercoagulable state. When the inner layer of the venous wall is damaged, it leads to localized blood cell aggregation and platelet adhesion, thereby forming a thrombus; Sluggish blood flow caused by prolonged bed rest, fracture pain, or post-anesthesia muscle relaxation promotes thrombus formation. A hypercoagulable state refers to blood's tendency to clot easily due to interactions among vascular endothelial cells, platelets, thrombin, anticoagulants, and the fibrinolytic system. Hypercoagulation increases the risk of platelet and

coagulation factor aggregation, thereby elevating thrombus formation risk. Metabolomics, from a microscopic perspective, utilizes analytical methods to identify low-molecular-weight compounds associated with VTE. This reveals that the pathogenesis of VTE may be linked to abnormalities in lipid metabolism, amino acid metabolism, energy metabolism, and adenosine metabolism.

**4.1 Lipid Metabolism**

Yeji Sung et al. [6] identified characteristic serum profiles in VTE mouse models. Using LM-CS and other analytical techniques, they detected significant differences in numerous lipids, primarily including sphingomyelin, phosphatidylcholine, and triglycerides. Yao Lv's research similarly revealed marked lipid differences among differential serum metabolites in patients with pregnancy-associated VTE [7].

1) Sphingomyelin Yeji Sung et al. [6] noted that sphingomyelin serves as a key metabolite distinguishing DVT from controls, linked to ceramides on the venous wall. Ceramides on the venous wall are central participants in sphingomyelin metabolism. Sphingomyelin is hydrolyzed by sphingomyelinase, yielding ceramides and phosphatidylcholine (PC). The role of ceramides in vascular inflammation has been well-established. Ceramides induce endothelial cells to release Weibel-Palade bodies [8,9], thereby promoting E-selectin-dependent adhesion of leukocytes to endothelial cells. These leukocytes promote inflammation and venous thrombus formation [10]. Animal studies have observed decreased plasma sphingomyelin in pigs with acute PE [11], while elevated levels of several sphingomyelin species were detected in serum and vein walls of DVT mouse models [12]. Human studies revealed elevated sphingomyelin in acute pulmonary embolism (APE) but reduced levels in chronic thromboembolic pulmonary hypertension [13]. These findings indicate sphingomyelin exhibits distinct patterns in thrombosis, suggesting potential as a metabolite for early VTE diagnosis.

2) Phosphatidylcholine Ming Xie et al. [14] identified key roles for phospholipid metabolism in APE pathogenesis through non-targeted metabolomics analysis. Notably, reduced PC levels and abnormal derivatives (e.g., lysophosphatidylcholine) may correlate with inflammation. Comparative studies between APE and non-ST-segment elevation myocardial infarction (NSTEMI) revealed elevated white blood cell and neutrophil counts in APE patients, reflecting systemic inflammation. Inflammation plays a crucial role in thrombogenesis by causing vascular endothelial injury, releasing procoagulant substances, and activating the coagulation system, ultimately leading to thrombus formation. Furthermore, thrombi can stimulate inflammation, leading to platelet release, aggregation, and adhesion of leukocytes (e.g., neutrophils) [15,16]. Phosphatidylcholine (PC), a major component of pulmonary surfactant [17], is implicated in the development and progression of pulmonary injury during APE through increased release of inflammatory mediators and activation of phospholipases. Phospholipases initiate inflammation, phosphorylation, and arachidonic acid metabolism, and their activation directly contributes to pulmonary injury. Using PC and other phospholipids as substrates, they degrade fatty acids at the sn-2 position, producing lysophosphatidylserine and free fatty acids. This leads to decreased PC levels, ultimately impairing alveolar function. In animal models, reduced phospholipid choline levels in venous walls were observed in DVT mice, while decreased plasma choline levels were noted in newly diagnosed VTE patients [12,18]. However, elevated PC levels were found in a rabbit jugular vein thrombosis model [19]; additionally, increased levels of specific lysophosphatidylcholine (LPC) were observed in the serum of DVT model mice [18]. These findings suggest that PC may not only serve as a potential diagnostic biomarker for VTE but that its metabolic pathway could also become a target for novel anticoagulant therapies.

**4.2 Amino Acid Metabolism**

In their metabolomics study of VTE and its chronic sequelae, Zahra Amirsardari et al. [20] searched PubMed, Embase, Scope US, Web of Science. Multiple studies highlighted alterations in serum amino acid and derivative levels in VTE patients, including: - Biosynthesis of valine, leucine, and isoleucine - Metabolism of alanine and aspartate - Metabolism of D-glutamine and D-glutamate - Metabolism of arginine

4.2.1 Tryptophan

Abnormal tryptophan metabolism, specifically through the dysregulated activation of the kynurenine pathway, is closely associated with venous thromboembolism (VTE). Compared to healthy controls, patients with chronic thromboembolic pulmonary hypertension (CTEPH) exhibit lower tryptophan levels and higher kynurenine levels [20]. In critically ill patients or those with chronic kidney disease (CKD) [20,21], early-stage tryptophan metabolism is preserved and persists in CKD patients, becoming residual urinary solutes [22,23]. These solutes include indoleacetic acid (IAA), l-kynurenine (Kyn), uric acid (UA), o-aminobenzoic acid (OABA), and xanthuric acid (XA) [19]. Among these, kynurenine increases vascular wall components via the uremic solute-AHR-TF thrombosis axis. Elevated levels of kynurenine and indole-3-carboxylic acid sulfate, metabolites of the tryptophan pathway, correlate positively with thrombus size [24].

4.2.2 Essential Amino Acids

Lucas G. Martins [25] noted the role of essential amino acids in thrombogenesis. Essential amino acids—valine (Val), leucine (Leu), and isoleucine (Ile)—play critical roles in regulating platelet activation. The catabolic breakdown of essential amino acids in the presence of multiple enzymes produces branched-chain ketone amino acids (BCKAs) and acyl-CoA. The ketone metabolites of valine and isoleucine (BCKAs) play a more significant role in enhancing platelet activation than those of leucine. Furthermore, acyl-CoA is the sole metabolite of the ketone amino acids valine and isoleucine, and it increases platelet activation. It also serves as a donor molecule for protein acylation, and enhanced acylation of cytoskeletal proteins is associated with upregulation of platelet activation. Moreover, elevated levels of essential amino acids and fatty acids can disrupt glycolysis and the TCA cycle by inhibiting pyruvate dehydrogenase

activity and suppressing succinate dehydrogenase gene expression.

### 4.3 Energy Metabolism

Multiple studies indicate that venous thromboembolic disease is associated with energy metabolism. Beata Franczyk et al. [2] proposed that the most described metabolites potentially related to VTE include carnitine, glucose, phenylalanine, 3-hydroxybutyrate, lactate, tryptophan, and several monounsaturated and polyunsaturated fatty acids. Furthermore, cellular responses to acute PE involve uncoupling between glycolysis and oxidative phosphorylation. A metabolomics study in PE patients revealed elevated TCA cycle levels—including malate, fumarate, isocitrate, α-ketoglutarate, and cis-aconitate—in high- and intermediate-risk acute PE patients compared to low-risk patients [26]. Yeji Sung et al. [6] identified decreased TCA cycle intermediates—citrate, succinate, succinyl-CoA, and fumarate—in serum from DVT mouse models in 2018. Although the underlying energy metabolism mechanisms remain controversial, studies demonstrate decreased TCA cycle intermediate levels in DVT mouse serum, indicating disrupted TCA cycle turnover. One potential factor for this disruption is reduced availability of acetyl-CoA. Acetyl-CoA has two primary sources: glycolysis and fatty acid metabolism involving carnitine. Lactic acid, a glycolysis-derived molecule, showed no significant difference in this study, whereas carnitine fatty acid metabolites exhibited a marked increase. Carnitine converts activated free fatty acids into acylcarnitine and shuttles acylcarnitine into mitochondria. Acylcarnitine is subsequently converted to acyl-CoA, which undergoes oxidation to form acetyl-CoA entering the TCA cycle. Additionally, Jie Cao [27] observed changes in acetoacetate (ketone body) and pyruvate levels similar to those seen in acute PE pig models. Elevated concentrations of these two metabolites suggest potential associations between ketone bodies and glucose metabolism with DVT, possibly linked to hypoxia-mediated alterations and carbohydrate metabolism.

### 4.4 Adenosine Metabolism

Research findings by Yeji Sung et al. [6] indicate that adenosine is the most prominent metabolite. Its concentration increased 9.5-fold in venous occlusion while decreasing 2.2-fold in serum. One explanation for the serum adenosine reduction is that decreased serum adenosine levels during thrombosis result from increased endothelial cell uptake coupled with reduced intracellular catabolism. Kazunari Maekawa et al. noted that among 226 metabolites, the 18 most significantly altered included glycolytic metabolites, redox-related metabolites, purine nucleotides, and tryptophan metabolites. Lactate and adenosine monophosphate (AMP) inhibit collagen-induced platelet aggregation. Adenosine is composed of adenosine nucleotides (adenosine diphosphate [ADP] and adenosine triphosphate [ATP]). During stress, hypoxia, or cellular injury, intracellular adenosine and ATP production are upregulated. The adenosine A1 receptor mediates vasoconstriction in the vascular bed [28,29], A2A and A2B adenosine receptors regulate vascular reactivity. Adenosine acting on these receptors mediates vasodilation, partly through direct effects on smooth muscle cells and partly via endothelial-mediated pathways. Beyond its direct vascular actions, A2A receptors also modulate local blood flow by reducing platelet aggregation and leukocyte adhesion to endothelial cells [30].

## 5. Conclusion

This review summarizes current advances in serum metabolomics research related to VTE, focusing on relevant metabolites and underlying mechanisms. However, numerous unresolved questions remain in VTE serum metabolomics. The potential diagnostic metabolites identified require validation through large-scale, multicenter clinical studies to establish their reliability.

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