



Glycosaminoglycans: Participants in Microvascular Coagulation of Sepsis

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Abstract

Sepsis represents a syndromic response to infection and frequently acts as a common pathway leading to fatality in the context of various infectious diseases globally. The pathology of severe sepsis is marked by an excess of inflammation and activated coagulation. A substantial contributor to mortality in sepsis patients is widespread microvascular thrombosis-induced organ dysfunction. Multiple lines of evidence support the notion that sepsis induces endothelial damage, leading to the release of glycosaminoglycans, potentially causing microvascular dysfunction. This review aims to initially elucidate the relationship among endothelial damage, excessive inflammation, and thrombosis in sepsis. Following this, we present a summary of the involvement of glycosaminoglycans in coagulation, elucidating interactions among glycosaminoglycans, platelets, and inflammatory cells. In this section, we also introduce a reasoned generalization of potential signal pathways wherein glycosaminoglycans play a role in clotting. Finally, we discuss current methods for detecting microvascular conditions in sepsis patients from the perspective of glycosaminoglycans. In conclusion, it is imperative to pay closer attention to the role of glycosaminoglycans in the mechanism of microvascular thrombosis in sepsis. Dynamically assessing glycosaminoglycan levels in patients may aid in predicting microvascular conditions, enabling the monitoring of disease progression, adjustment of clinical treatment schemes, and mitigation of both acute and long-term adverse outcomes associated with sepsis.

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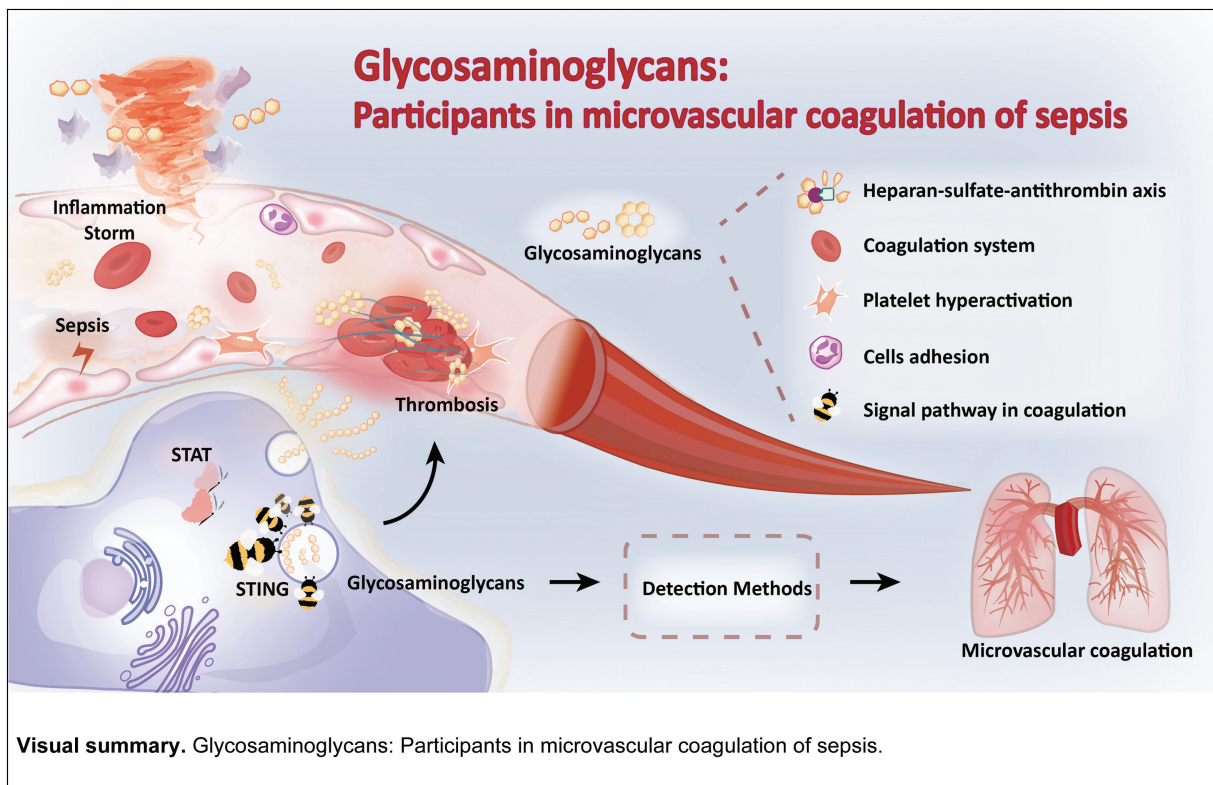
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Introduction

Sepsis is defined as a life-threatening organ dysfunction and is attributed to a dysregulated host response to infection in the new Sepsis-3 definitions.¹ It poses a significant global health threat and serves as a common pathway to death in cases of severe infectious diseases, particularly those manifested as pulmonary infections, which exhibit a high incidence rate and mortality in intensive care patients.² Annually, sepsis is responsible for approximately 20% of global deaths.³ The existing data are predominantly derived from developed countries with advanced medical infrastructure, potentially resulting in a higher mortality rate than currently reported, underscoring the urgency of addressing sepsis as a crucial health concern.⁴ Moreover, data obtained from numerous cohorts indicate that sepsis is the primary cause of death in the unprecedented outbreak of COVID-19 (coronavirus disease 2019).⁵⁻⁹ Consequently, to mitigate the annual deaths caused by infections worldwide, it is imperative to comprehend the underlying mechanisms and advance the development of detection methods throughout the sepsis process.

For decades, the extracellular matrix was regarded as an inert scaffold, serving functions such as providing essential elements for environmental support, mechanical support, and tissue protection.¹⁰ However, it is now acknowledged as a highly dynamic partner of the immune system, gaining significance in the field of sepsis. Recent reviews highlight the significance of the glycocalyx in microcirculation. The glycocalyx, constituting the extracellular matrix, is

composed of membrane-attached proteoglycans, glycosaminoglycans (GAGs), and other adherent plasma proteins. Near the plasma membrane, the membrane-tethered scaffold comprises syndecans, glypican proteoglycan families, and CD44, providing attachment sites for GAGs.¹¹ The negatively charged sulfated GAGs combine with plasma proteins such as albumin, fibrinogen, fibronectin, antithrombin (AT) III, and thrombomodulin. Additionally, unsulfated hyaluronan (HA) can form the complexes with sulfated GAG-containing proteoglycan, for example, versican.^{12,13} These GAGs not only aid in maintaining dynamic tissue integrity but also act as signaling molecules, actively participating in and driving biological processes.¹³

Moreover, prior studies have hinted at an undiscovered connection between the glycocalyx and thrombosis. Schmidt et al demonstrated that urinary indices of GAG fragmentation correlate with outcomes in patients facing critical illnesses such as septic shock or acute respiratory distress syndrome.¹⁴ Shalaby et al showed that endothelial dysfunctions exhibited by endothelial-derived microparticles possess procoagulant properties but elude detection through conventional coagulative tests.¹⁵ Stemming from these studies, we hypothesize that free GAGs released from the glycocalyx likely play a crucial role in coagulation during sepsis. Consequently, we have undertaken a comprehensive review of the research advancements related to GAGs, with a specific focus on their involvement in sepsis-induced thrombosis dysfunction and their potential role in promoting microthrombosis. Additionally, we delve into ongoing progress in detection methods.

Thrombosis, Inflammation, and Endothelial Cells in Microvasculature of Sepsis

The microvasculature, which includes first-order arterioles, first-order venules, and the capillary network, plays a crucial role in the functionality of tissues and organs. It regulates blood flow, vascular permeability, and acts as the principal site for gas and solute exchange between the bloodstream and tissues. The inner layer of the microvasculature consists of closely connected endothelial cells, which represent one of the initial cell types to encounter and respond to insults by amplifying the immune response and the coagulation system.

In the state of sepsis, several complex factors are involved in and contribute to the formation of microvascular thrombosis. These factors include the direct role of certain pathogens, the activation of the plasma coagulation cascade, activated platelets, injured endothelial cells, and the activated complement system by pathogens.^{16–19} Bacteria can promote platelet activation and aggregation, thereby exacerbating inflammation and coagulation reactions, ultimately leading to the microcirculation thrombosis.

Endothelial cells are excessively stimulated by pathogens and a large number of host-derived infection mediators, which damage the glycocalyx. The degradation of the glycocalyx increases the exposure and expression of molecules from endothelial cells, including adhesion molecules, growth factors, and cytokines. This, in turn, results in the accumulation of leukocytes, erythrocyte networks, and stacks. Leukocytes, such as neutrophils, macrophages, and eosinophils, release neutrophil extracellular traps (NETs). NETs are extracellular, web-like decondensed nuclear or mitochondrial DNA structures composed of histones, cytosolic and granule proteins.^{20–22} Notably, neutrophils and NETs stimulate pro-inflammatory and pro-angiogenic responses in endothelial cells, causing further dysregulation in both innate and acquired immune systems.^{23–25}

Moreover, NETs can also serve as a scaffold for both thrombosis and complement activation. The complement system, a crucial component of the innate immune system, plays a significant role in seeking and defending against pathogen invasion. The activation of the complement system is closely related to promoting inflammation and activating the coagulation cascade reaction. For example, complement C5 is considered as an unconventional procoagulant molecule and is associated with complement activators, such as thrombin and damaged endothelium.²⁶ The neutrophil-complement-coagulation system has been shown to facilitate the formation of microthrombi and clots in the microvasculature.^{27–29}

Particularly, when pathogens invade the vascular system, pattern recognition receptors (PRRs) within the innate immune system, consisting of neutrophils, monocytes, natural killer cells, among others, are triggered by binding to pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns.^{30–32} The PRRs activate the Toll-like receptors (TLRs) and nucleotide-binding domain leucine-rich repeat-containing protein inflammasome signal

transduction, ultimately prompting the transcription of proinflammatory factors in innate immune cells.^{33–36} These proinflammatory factors also play a crucial role in activating endothelial cells, innate immune cells, and platelets, thereby contributing to coagulation pathways aimed at initially containing the infection.^{37–40} This initial controlled vascular response, during minor infections, represents an immune-protective effect coordinated by the tissue response to local infection. However, in severe sepsis, this vascular response becomes overactivated, resulting in an inflammatory storm that damages the vascular endothelium.⁴¹ Recent research studies have demonstrated that the adhesion of pathogens to host cells relies on the mediation of GAGs.^{42,43} The hyperactivation of innate immune cells and endothelial cells dysregulates the glycocalyx barrier, giving rise to systemic microvascular thrombosis.⁴⁴ This, in turn, causes the accumulation of leukocytes, impaired perfusion, and albumin filtration, ultimately accelerating the progression of multi-organ dysfunction.^{22,45–48}

Nevertheless, it is essential to highlight that microvascular inflammation, as mentioned earlier, is not the sole contributor to coagulation disorders in the host during sepsis. Common bacteria such as staphylococci and streptococci are equipped with proteins capable of directly interfering with the human coagulation cascade or the fibrinolytic system. The interaction between staphylocoagulase and prothrombin results in the formation of staphylothrombin, independently inducing coagulation without reliance on other vascular (cellular) mechanisms of coagulation activation. Additionally, staphylokinase and streptokinase can dysregulate fibrin through the activation of fibrinolytic cascades, representing another cause of coagulation dysfunction in bacteria sepsis for the host.^{49–51}

The Structures and Physiological Functions of GAGs

GAGs are linear, highly charged, and heterogeneous acidic polysaccharides expressed in various types of cells. Their backbones are regular and consist of repeating disaccharides with alternating uronic acid (UA)/galactose (Gal) and hexosamine (HexN) units.⁵² GAGs can be categorized into four groups based on the combinations of units, sulfation patterns, and residues: heparan sulfate (HS)/heparin (Hp), chondroitin sulfate (CS)/dermatan sulfate (DS), keratan sulfate, and HA. Most GAGs are sulfated and attached to core proteins (e.g., syndecans 1–4 and glypicans 1–6), except for HA, which is attached to the receptor CD44.¹¹

GAGs exhibit two main characteristics: combination diversity and electronegativity. The combinations of disaccharides occur randomly, stemming from their non-template-driven nature, which is different from DNA semi-conservative replication. Previous research has demonstrated that six disaccharide units can theoretically form 12 billion different GAGs. This number is significantly larger than the combinations of nucleic acids and peptides, implying their potential for biological diversity.⁵³ Furthermore, the degree of sulfation in GAGs is linked to various diseases.⁵⁴

GAGs act as sieves to limit the passage of molecules with the same charge or those larger than 70 kDa due to their negatively charged characteristic. The negatively charged sulfate groups in GAGs provide binding and charge-neutralizing sites for proteins with positively charged or polar residues, thereby altering their structures and determining their functions. This suggests that GAGs may play a role in regulating signal transductions by (1) acting as activators that mediate the formation of signal complexes, (2) serving as repressors, (3) functioning as concatenators, and (4) acting as selectors that favor the formation of one complex over another.^{55,56}

Under physiological conditions, the intact structure of the glycocalyx and GAG components play a crucial role in regulating cell adhesion and maintaining vascular homeostasis. GAGs mediate cell adhesion by acting as mechanosensors, regulator of nitric oxide (NO) production, and barriers to inhibit cell adhesion. NO, a vasodilator and anti-atherogenic molecule, is primarily regulated by vascular shear stress. NO and endothelial NO synthase (eNOS) are commonly used indicators to define endothelial cell dysfunction.

In the vascular system, sustained directed mechanical forces contribute to maintaining vascular homeostasis, while the mechanical forces lacking a definitive direction result in sustained molecular signaling of pro-inflammatory pathways.⁵⁷ GAGs, in collaboration with other mechanosensors such as syndecans, PECAM-1, and $\alpha_5\beta_1$, function as mechanotransducer signal platforms.^{58,59} They transduce mechanical stimuli, leading to the production of NO, supporting the overall functions of endothelial cells.⁶⁰ Particularly, when the HS structure is preserved, eNOS can be upregulated to produce NO.⁶¹ Therefore, in the context of minor inflammation and coagulation, intact endothelial cells with a preserved glycocalyx can generate NO. This inhibits further platelet activation and leukocyte adhesion to the endothelium, thereby maintaining the local vascular homeostasis.^{62,63}

Biosynthetic Pathways of GAGs

GAGs, whether existing independently or in conjunction with proteins,⁶⁴ are extensively distributed throughout the extracellular matrix, cell surface, and cytoplasm. The specific sites for GAG synthesis are intricately determined by the presence of particular enzymes and precursors. Sulfated GAGs are synthesized within the Golgi apparatus, and their extension relies on attachment to the core protein linkage oligosaccharides with a glucuronosyl-galactosyl-galactosyl-xylosyl tetrasaccharide structure, which is linked to serine residues of the core proteins.⁶⁵ Subsequently, these proteoglycans are excreted into the extracellular matrix or localized on the plasma membrane. Following this, the necessary glycosyltransferases and other enzymes, provided by the Golgi apparatus, elongate and modify the sulfated GAG chains through processes such as epimerization and sulfation.⁶⁶

Unlike sulfated GAGs, HA undergoes self-elongation without the assistance of anchor proteins. Its synthesis occurs at

the inner plasma membrane, where the essential initial materials, including UDP-GlcNAc (uridine diphosphate-N-acetylglucosamine) and UDP-GlcUA (uridine diphosphate-glucuronic acid), as well as HA synthase enzymes (HAS1, HAS2, and HAS3) are present. Among HA synthase enzymes, HAS2 serves as the primary HA synthase, responsible for the production of HA. HAS3, on the other hand, exhibits high expression under specific conditions.⁶⁷ Following its synthesis, HA is directly secreted into the extracellular matrix after undergoing modifications.⁶⁸

The Golgi apparatus exhibits a fascinating phenomenon by acting as a central hub for the synthesis of sulfated GAGs. Additionally, it serves as the activation site for the stimulator of interferon genes (STING), an immune adaptor protein associated with the endoplasmic reticulum (ER). STING plays a crucial role in initiating and amplifying inflammatory responses to PAMPs.^{69,70} This unique occurrence opens up numerous possibilities for the interaction between STING and GAGs. Our laboratory conducted molecular docking experiments to simulate and investigate the *in vitro* affinity between GAGs and STING. Furthermore, the interaction with STAT was examined, which is another significant immune regulatory protein responsible for transducing cytokine signals from the cell membrane to the nucleus following phosphorylation by Janus kinases. Results revealed that STAT1, STAT4, STAT3, and STAT6 can mediate cell death during sepsis.^{71,72} Additionally, robust interactions between GAGs and both STING and STAT *in vitro* were revealed. These findings established a foundation for studying the potential molecular pathways through which GAGs may be involved in the microcoagulation associated with sepsis.

The Degradation of GAGs in Sepsis

During sepsis, the functionality of endothelial cells becomes compromised, and the structural integrity of the glycocalyx is disrupted, leading to the degradation and shedding of a substantial amount of GAGs. The initiation of GAG degradations is triggered by the activation of enzymes, lysosome impairment, and the generation of reactive oxygen species (ROS).⁷³ Several enzymes play a role in this degradation process, including heparinase, hyaluronidases, and matrix metalloproteinases (MMPs). Heparinase, found in mastocytes and platelets, induces the cleavage of HS chains attached to core proteoglycans.^{74,75} MMPs present in vascular endothelial cells and macrophages cleave proteoglycans from the endothelial cell membrane.⁷⁶ Neutrophilic granulocytes house proteolytic enzymes such as serine proteases elastase and proteinase-3, which can shed the HA by cleaving the binding of the HA-receptor CD44 complex.⁷⁷ Endothelial cells themselves contain hyaluronidases that cleave the HA into tetrasaccharides.^{78,79} Beyond the enzymatic degradation, various cell types, including endothelial cells, platelets, and neutrophils, can be induced to release ROS by activated TLRs during sepsis, contributing to the further degradation of GAGs.⁴⁴

Several degradation components of glycocalyx have been identified as valuable biomarkers for endothelial cell damage

in sepsis. Among these components, Syndecan-1, a type of proteoglycan, has garnered significant attention, being the foremost glycocalyx shedding component in sepsis.⁸⁰ Numerous studies have established a significant correlation between the plasma Syndecan-1 levels in the early stages of sepsis patients and the severity and incidence of late-stage organ failure. This correlation proves to be instrumental in predicting the development and prognosis of the patient, thereby establishing Syndecan-1 as a biomarker for clinical adjuvant diagnosis and treatment of sepsis.^{81,82}

Another promising biomarker for the degradation components of glycocalyx is the shedding GAGs.⁸³ Recent studies have indicated notably higher plasma levels of free HS or HA in sepsis patients or model animals compared with healthy individuals. Importantly, these elevated levels have been found to be associated with severity of the subjects' condition.^{14,84} Given the diversity of types and combinations of GAGs, assessing the quantitative level and composition of shedding GAGs in patients may offer richer information and data for clinical prediction, diagnosis, and treatment of sepsis, warranting further research.

GAGs and Coagulopathy

Recent research has increasingly demonstrated a strong connection between thrombosis and inflammation through immunothrombosis, revealing platelets and innate immune cells as the main cellular drivers of this process.^{44,85} Although the precise trigger mechanism remains unclear, GAGs may play a significant role, primarily through two mechanisms: (1) upregulating the procoagulant pathway by activating the contact system, enhancing cell adhesion, and inhibiting kallistatin; and (2) downregulating physiological anticoagulants by interfering with AT activation and inhibiting tissue factor pathway inhibitor (TFPI). This, in turn, further activates the microthrombotic pathway and amplifies inflammation.

GAGs and Coagulation System

The contact system is an intrinsic coagulation system responsible for inducing a hypercoagulable state in septic patients. Factors XIa (FXIa), XIIa (FXIIa), and plasma kallikrein (PKa) of the contact system of coagulation appear to contribute to thrombosis.⁸⁶ Traditionally, the contact system and the tissue factor (TF) pathway are considered mutually independent main coagulation pathways.⁸⁷ Additionally, the traditional perspective holds that GAGs are primarily recognized for their anticoagulant properties, such as the high-affinity Hp, which can stimulate the inhibition of several coagulation enzymes by interacting with AT and Hp cofactor II (HC II).⁸⁸ However, recent evidence showed that endogenous negatively charged GAGs can also activate the contact system in normal human plasma.⁸⁹ The abnormal generation of GAGs from diverse sources, exhibiting varying degrees of sulfation, encompasses chemically oversulfated GAGs, GAGs produced by tumors, and the IgG/PF4/Hp complexes. These entities collectively contribute to thrombin generation by activating the contact system. In addition, the IgG/PF4

complex stimulates platelets, subsequently initiating coagulation on the negatively charged surface of activated platelets.

Platelets, anucleate blood cells originating from megakaryocytes, play fundamental roles in coagulation as they engage with endothelial and leukocytes cells. During sepsis, these cells undergo activation, releasing chemokines, inflammation mediators, and microparticles. PF4, a Hp-binding protein with specific procoagulant activity, is released from stimulated mature platelets.⁹⁰ Upon release, PF4 binds to GAGs, forming an antigenic complex.^{91,92} In patients with severe sepsis facing a dual risk of Hp-induced thrombocytopenia and thrombosis, IgG combines with PF4. This complex then binds to platelet Fcγ receptors, inducing platelet activation and aggregation.⁹³ Additionally, PF4 has the capacity to neutralize the negative charge of GAGs. This action facilitates the adherence of negatively charged platelets to the endothelium, thereby promoting thrombus formation with increased efficacy.⁹⁴

Hayes et al utilized confocal microscopy to illustrate that PF4, released from activated platelets, binds to surface GAG side chains on intravascular and vascular cells. Furthermore, PF4 adheres more effectively to the peri-injury endothelium, characterized by a glycocalyx rich in high-affinity HS and DS.⁹⁵

TFPI, which binds to HS, acts as a negative regulator of the extrinsic coagulation pathway. It accomplishes this by downregulating coagulation function through interactions with TF-factor VIIa and factor Xa. Additionally, TFPI plays a crucial role in coagulation regulation by augmenting the inhibition of factor Xa and decreasing prothrombinase activity. The shedding of HS is shed from endothelial cells, disrupting TFPI and leading to coagulation.⁹⁶

Kallistatin, a serpin, exerts its inhibitory effect on kallikrein by binding with GAGs. This interaction ultimately triggers the activation of factor XII and the subsequent cleavage of high-molecular-weight kininogen into bradykinin. Both processes contribute to a pro-coagulation effect.^{97,98}

GAGs and Cell Adhesion

GAGs located on the surface of endothelial cells play a pivotal role in maintaining the antithrombotic properties of the vascular system. In the context of sepsis, GAGs are susceptible to disruption caused by ROS, heparanases, and various proteases. This disruption leads to the exposure of adhesion molecules, particularly E-selectin is exposed to the endothelial cell surface, subsequently promoting the recruitment of platelets and leukocytes. Chaaban et al demonstrated that HA with a molecular weight below 1,000 has a significant activating effect on histone-induced platelet aggregation.⁹⁹ The degradation of GAGs also hampers the responses of endothelial cells to shear stress and exacerbates adhesion, resulting in thrombotic events.^{22,100}

Platelet endothelial cell adhesion molecule-1 (PECAM-1) and heterotrimeric C protein subunits Gαq and 11 (Gαq/11) act as mechanosensors that respond to shear stress and mediate downstream signals. Dela Paz et al found that intact HS on endothelial cells promotes the formation of a

complex between these two proteins and its removal attenuates flow-induced Akt phosphorylation.¹⁰¹ Additionally, Schabbauer et al demonstrated that inhibiting Akt enhances lipopolysaccharide-induced coagulation and inflammation.¹⁰² Both findings suggest that the shedding of HS may induce clotting.

GAGs and Signal Pathway in Coagulation

The central innate immune cells are responsible for initiate both inflammatory and coagulant responses.^{103,104} Excessive activation of these host innate immune and coagulation responses has been linked to multi-organ failure and death.⁴ As mentioned earlier, STING plays a crucial role in initiating and magnifying inflammatory responses to PAMPs. Research has shown that GAGs can regulate STING in immune pathways, and the overactivation of STING is closely associated with sepsis. Zhang et al revealed that STING drives coagulation by initiating ER stress, leading to the activation of gasdermin D (GSDMD), an effector of pyroptosis. This process subsequently releases TF, triggering the coagulation cascade in sepsis patient samples, mice, and cell models.¹⁰⁵ In a related study, Fang et al identified that in vitro, the interaction

between STING and sulfated GAGs further promotes the polymerization of STING through electrostatic attractions between negatively charged sulfate groups of GAGs and positively charged amino acids of STING.¹⁰⁶ Additionally, Chen et al demonstrated that STING is essential for the virus-induced activation of STAT6, members of the signal transducer and activator of transcription family, in vitro.^{71,107,108}

► **Fig. 1** summarizes potential pathways. Interestingly, GAGs can bind to STING, but the binding site, kinetics, downstream effects, and the role of negative charge in this interaction remain unknown. Further studies are needed to explore these aspects, as they may reveal one of the potential pathways by which GAGs are involved in clotting.

IL-27 activates the STAT pathway and regulates immune responses, particularly STAT1 and STAT3.¹⁰⁹ Cavé et al proved that GAGs bind to human and mouse IL-27, thereby regulating the activation of STAT1 and STAT3.¹¹⁰ Furthermore, STAT3 mediates endothelial dysfunction and plays a key role in sepsis-induced multiple organ failure by inducing disseminated intravascular coagulation.^{71,111} Beckman et al, using vascular endothelial cells, found that JAK-STAT inhibition limited the secretion of pro-adhesive and procoagulant

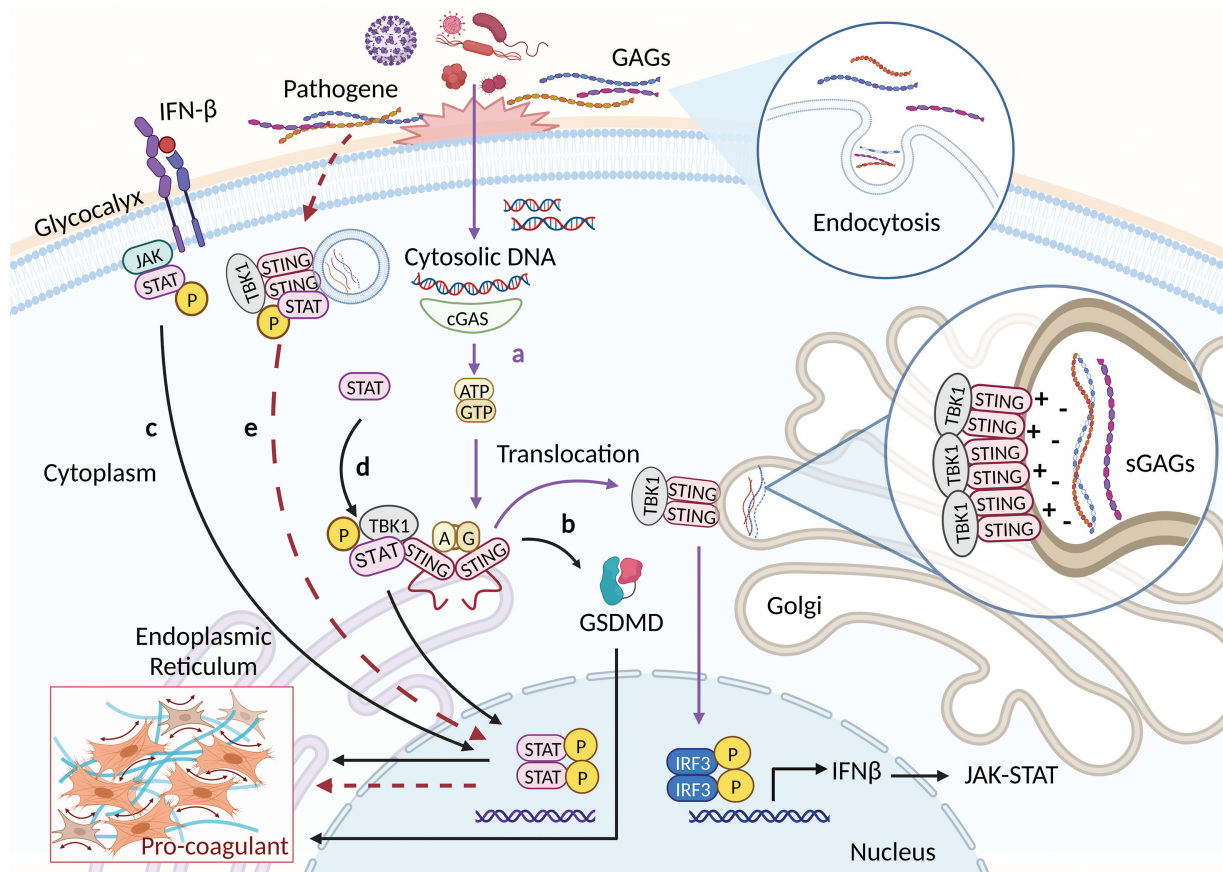


Fig. 1 Summary of potential signaling mechanisms of thrombosis induced by glycosaminoglycans. Summary of potential signaling mechanisms of thrombosis induced by GAGs. (A) Sulfated GAGs in the Golgi apparatus or vesicles directly drive the STING polymerization and activation through electrostatic attractions. (B) STING-mediated cleavage of GSDMD triggers the release of tissue factor F3, contributing to coagulation. (C) STAT2 and STAT3 control the expression of endothelial adhesion molecules, initiating endothelial dysfunction during sepsis and subsequently participating in coagulation. (D) Viruses or cytoplasmic nucleic acids trigger STING to recruit STAT6 to the endoplasmic reticulum, leading to STAT6 phosphorylation independent of JAKs. (E) Apart from the classical STING and STAT pathways, sulfated GAG fragments damaged by various infectious factors move into immune cells or endothelial cells by phagocytosis, activating STING and STAT to regulate coagulation. (Created in BioRender.com). GAGs, glycosaminoglycans.

factors. It also reduced endothelial TF and urokinase plasminogen activator expression.¹¹² These imply that GAGs may regulate the STAT pathway by binding with IL-27, ultimately influencing coagulation.

In essence, GAGs regulate the activities of enzymes, chemokines, cytokines, and growth factors through binding with proteins.^{55,113,114} However, their mechanisms of operation and their role in coagulation remain unclear. The aforementioned pathways may contribute to our understanding of GAGs signaling pathways.

GAGs and Hp/HS–Antithrombin Axis

Thrombin serves as the final protease generated in the blood coagulation cascade, responsible for cleaving fibrinogen and forming the fibrin clot. AT, a significant plasma glycoprotein belonging to serpin superfamily, acts by inhibiting thrombin and activating factor X. It achieves its anticoagulation role through interactions with GAGs.¹¹⁵ HC II also inhibits thrombin activity to facilitate anticoagulation.¹¹⁶ Both AT and HC II bind to heparan through a pentasaccharide or hexasaccharide sequence, respectively, inducing conformational changes in the reactive center loop of the serpin. These changes enhance the activity of serpin, contributing to its anticoagulant properties.¹¹⁵ Simultaneous binding to AT and thrombin requires a minimum chain length of 18 saccharides, while binding to HC II and thrombin requires 30 saccharides.¹¹⁵ The longer the Hp, the greater chance of specific pentasaccharide sequences appearing and an increased number of negative charges are more likely to accumulate which will enhance the anticoagulant effect.

However, during the initial phases of sepsis, numerous glycosidases are released into the bloodstream, causing the breakdown of GAGs. For example, heparanase is activated by inflammatory cytokines and ROS, resulting in the degradation of Hp. Although the low-affinity Hp which without specific sequences maintains its AT activity, its affinity diminishes in comparison to that with specific sequences, rendering it incapable of executing anti-activated factor X activity.¹¹⁷

Additionally, products produced by pathogens can bind to GAGs, inhibiting the Hp-dependent anticoagulant function of AT and promoting coagulation pathways. Concurrently, they exert a proinflammatory effect. Histidine-rich protein II (HRPII), a protein exclusively produced by *Plasmodium falciparum*, binds to GAGs to prevent their interaction with AT and FXa or thrombin in vitro.^{118,119} Dinarvand et al demonstrated that HRPII may also interact with the AT-binding vascular GAGs, thereby inhibiting the anti-inflammatory signaling function of the serpin.¹²⁰ Inflammatory stimulation has been shown to downregulate and impair GAGs in endothelial cells, leading to a decrease in the effective binding between GAGs and AT.^{121,122} In a study by Kobayashi and colleagues, porcine aortic endothelial cells were pretreated with IL-1 β or rTNF α , resulting in suppressed HS synthesis and a subsequent reduction in binding of AT III to the cell surface.¹²³ Moreover, it has been noted that plasma fibronectin maintains a compact conformation while circulating in the bloodstream. However, upon binding to GAGs, its structure undergoes a transformation into an

extended conformation, forming fibrils that disrupt the interaction between GAGs and AT.¹²⁴

Detection Methods of GAGs

Detection methods for GAGs in vivo, particularly quantitative analysis techniques, are crucial for a comprehensive exploration of the mechanisms through which GAGs contribute to coagulation disorders in sepsis. These methods are also vital for early prediction and dynamic monitoring of disease progression and prognosis in sepsis patients. Additionally, they play a key role in anticipating when alternative treatment strategies can be applied. Currently, the predominant method employed in clinical settings is the imaging detection. Notably, recent advancements have been made in achieving precise and rapid quantification of GAGs. In contrast to the widely used imaging detection methods for GAGs, liquid chromatography-mass spectrometry (LC-MS) quantitative technology stands out. This approach not only enables accurate quantification of different GAG disaccharide components but also facilitates qualitative analysis of the composition of GAGs. This dual capability is constructive for simultaneously analyzing changes in both the content and composition of GAGs in sepsis patients.

Imaging Detection of GAGs

As the primary line of defense for blood vessels, the endothelial glycocalyx exhibits a thickness ranging from 200 to 2,000 nm.¹²⁵ Current imaging detection methods face limitations in directly identifying GAGs but instead measure the overall thickness of glycocalyx, as these methods struggle to distinguish the individual components of the glycocalyx. Researchers have observed dissociative glycocalyx using immunogold staining through transmission electron microscopy.^{126–128} Another experimental approach is scanning electron micrographs, offering a three-dimensional representation of glycocalyx coverage.^{125,129,130} In clinical research, noninvasive microscopic camera techniques like sidestream dark field (SDF) imaging and orthogonal polarization spectral (OPS) have been applied to measure and visualize glycocalyx damage.^{131,132} However, these methods are susceptible to various factors, including differences in thickness algorithms and observation discrepancies between in vivo and in vitro conditions.¹³³ To overcome these challenges, a novel analysis software named GlycoCheck was developed, specifically designed to work with two main SDF devices (Microscan and CapiScope HVCS). This software aims to standardize the results obtained from both methods.¹³⁴ Furthermore, Xiao et al reported the use of PLL-MNPs, positively charged nanoparticles that can selectively target GAGs through electrostatic interactions, revealing the relationship between GAG components and progression of osteoarthritis.¹³⁵

The general advantage of these imaging methods lies in their ability to directly depict the shedding of GAGs or reflect their damage through microvascular visualization, rendering them noninvasive. However, they fall short in accomplishing both quantitative and qualitative analyses of distinct and individual GAG components.

Nonvisual Detections of GAGs

Currently, LC-MS, nuclear magnetic resonance,^{136,137} enzyme-linked immunosorbent assay,^{138,139} and chemometric analysis¹⁴⁰ are commonly used nonvisual methods for quantifying

GAGs.¹⁴¹ ► **Table 1** lists the commonly used visual and nonvisual methods for GAG detection, including biological sample types and the associated disease. Bio-layer interferometry (BLI) is employed to detect intermolecular interactions and

Table 1 Summary of samples in detection methods of GAGs

Detection methods	Object	Sample	Diseases	Ref.
TEM	HA	Extraocular muscles (human)	Thyroid-associated ophthalmopathy	126
	Glycocalyx	Implanted homograft valves (human)	Cardiac homograft valve implantation	127
	Glycocalyx	Hearts (guinea pig)	Ischemia/reperfusion	128
SEM	Glycocalyx	Male Sprague-Dawley rats	Cardiac arrest and cardiopulmonary resuscitation	125
	Glycocalyx	Umbilical cord postpartum (human)	Healthy	129
	Glycocalyx	Brains, hearts, lungs (mice)	Healthy	130
SDF	Glycocalyx	Human	Emergency room	134
OPS	Glycocalyx	Human	Healthy	131,132
NMR	GAGs	Thymus, brain, kidney (mice)	Healthy mice and mice with genetic alterations in glycosyltransferases	137
	GAGs	Solvents	–	136
ELISA	HA HS	Serum (SD rats)	Cardiac arrest and cardiopulmonary resuscitation	125
	HS	Arterial blood (human)	Coronary artery bypass graft surgery with bypass	138
Chemometric analysis	CD/DS and HS	Urinary (human)	Systemic sclerosis	140
LC-MS	GAGs	Samples from human and animals	–	144,159,164–171

Abbreviations: ELISA, enzyme-linked immunosorbent assay; GAGs, glycosaminoglycans; LC-MS, liquid chromatography-mass spectrometry; NMR, nuclear magnetic resonance; OPS, orthogonal polarization spectral; SDF, sidestream dark field; SEM, scanning electron microscopy; TEM, transmission electron microscopy.

Table 2 Bio-layer interferometry methods of GAGs

	Biosensors	Binding protein	Instrument	Ref.
1	Streptavidin biosensors (ForteBio)	Heparin AND binding PF4	Octet Red96 system (ForteBio)	159
2	Sensor streptavidin (SA) chips were from GE Healthcare (Biacore AB, Uppsala, Sweden)	Tissue inhibitor of metalloproteinases-3 (TIMP-3) AND biotinylated heparin was immobilized to the streptavidin (SA) chip	BIACore 3000	160
3	SA sensor chips were from GE Healthcare (Uppsala, Sweden)	The biotinylated HP was immobilized to streptavidin (SA) chip based on the manufacturer's protocol AND SARS-CoV-2 S-protein	BIACore 3000	161
4	Sensor SA chips were from Cytiva (Uppsala, Sweden)	The biotinylated GAGs were immobilized onto streptavidin (SA) chips AND Monkeypox Virus Protein A29	BIACore 3000 or T200 SPR (Uppsala, Sweden)	162
5	IASys auto plus device (Affinity Sensors, Cambridge, United Kingdom)	Biotinylated albumin-heparin was immobilized on an avidin-activated sensor chip AND hIL-10	–	163

Abbreviation: GAGs, glycosaminoglycans.

Table 3 LC-MS methods for analysis of GAGs

	Derivatization	pH	Samples	Labels	Time of separation	Ref.
1	Yes	–	Human urine	AMAC	15 min	144
2	Yes	5.6	Human serum, human red blood cells, human platelets, human granulocytes	AMAC	39 min	164
3	Yes	6.8	Cell cultures, liver tissue, urine	AMAC	60 min	165
4	Yes	–	Fish liver and intestines	1-Phenyl-3-methyl-5-pyrazolone (PMP)	7 min	166
5	Yes	5.6	Cell cultures	AMAC	–	159
6	Yes	4.4	Human IgG	2-Aminobenzamide (2-AB)	55 min	167
7	No	–	Heparin and heparan sulfate (pharmaceutical products)	–	8 min	168
8	No	–	Mouse tissues	–	20 min	169
9	No	Alkaline	Human synovial fluid	–	40 min	170
10	No	11	Porcine articular cartilage and yellow ligament	–	35 min	171
11	No	Acidic	Human urine	–	21 min	172
12	No	4.4	Tissue sections	–	20 min	173
13	No	–	Human brain	–	–	174
14	No	Acidic	Mouse tissues	–	~8 min	175

can provide relative affinity information between binding partners. ► **Table 2** presents a summary of recent studies utilizing BLI to assess the affinity between GAGs and proteins.

In this review, we focus on the rapid, sensitive, and accurate LC-MS method for its promising prospect in the study of GAGs in sepsis. This method allows for the fast quantification of multiple GAG components simultaneously and can analyze changes in GAG composition. As a result, it provides richer information for the clinical prediction, diagnosis, and treatment of sepsis.^{142,143} Numerous researchers have utilized LC-MS to measure GAGs in plasma and urine samples from both healthy and diseased adults.^{14,144–146} Li et al determined the average contents of HA, CS, and HS in 20 cell lines.¹⁴⁷ Furthermore, LC-MS offers a new strategy for analyzing the proteome that interacts with GAGs.^{148–150} Golden et al employed the LC-MS method to demonstrate the significant roles of HS and HA in neutrophil trafficking and subsequent pathological thrombosis in the liver vasculature of sepsis mice.¹⁵¹

The composition of disaccharides, commonly referred to as the GAGome, is impacted by the progression of diseases, particularly sulfated disaccharides. Various cells express diverse disaccharides, contributing to the formation of GAGs, which can serve as a foundational aspect for comprehending mechanisms underlying multi-organ dysfunction. LC-MS stands out from other methods as it not only quantifies concentrations of GAGs but also provides insights into the GAGome from a unique perspective.^{152,153} Here, we offer an overview of the literature related to the separation of GAGs based on LC-MS, presented in ► **Table 3**.

Additionally, MS can be utilized for offline determination of GAG sequences, providing essential data for elucidating precise structures, including carbohydrate chains and

modifications.^{154,155} The analysis of the GAGome, however, relies heavily on a robust database. To meet the growing demand for data analysis, numerous algorithms have emerged. MaatrixDB (<http://matrixdb.univ-lyon1.fr/>) is dedicated to biomolecular interactions involving extracellular matrix proteins and GAGs.¹⁵⁶ The GAGfinder was specifically designed to identify tandem mass spectrum peaks, addressing the issue of time-consuming analysis.¹⁵⁷ Duan et al developed a genetic algorithm approach to examine the theoretical structure of GAGs in its entirety, as opposed to constructing a structure from the ground up. This approach has been proven successful in examining both moderately sulfated GAGs and more highly sulfated GAGs.¹⁵⁸

Conclusion

This review highlights the significance of GAGs as crucial components within the intact glycocalyx, playing a key role in maintaining vascular microenvironment homeostasis. These functions include endothelial protection, serving as a selective permeability barrier for the vascular wall, and acting as vital shear stress receptors to prevent thrombosis and leukocyte adhesion. Pathological conditions, such as pathogen stimulation, can result in endothelial cell damage leading to the shedding of GAGs from the glycocalyx. The shed GAGs, in turn, serve as potent signaling molecules, participating in and driving the formation of immune microthrombosis in sepsis. This process ultimately contributes to the development of multiple organ dysfunctions in sepsis.

The potential role and significance of shedding GAGs in the formation of microcirculatory thrombosis in sepsis are gradually being discovered and studied, which is expected to

have a profound impact on deepening our understanding of the complex pathogenesis and clinical treatment of sepsis. Our laboratory has recently conducted research revealing a strong affinity between STING and STAT with GAGs in vitro, using molecular docking models. This finding suggests that shedding GAGs may induce the formation of microvasculature immune thrombosis in sepsis by activating STING, a major immune and inflammatory signaling pathway. Consequently, we have deduced the potential STING-mediated signaling pathways that interact with the shedding GAGs, hoping to provide new insights into the mechanism underlying sepsis thrombosis. This area of study merits increased attention and exploration. Additionally, our laboratory has made noteworthy progress in the LC-MS analysis of GAGs. This analytical method enables rapid and high-throughput detection without the need for sample derivatization pretreatment (the data are not displayed). We believe that the LC-MS methods for analyzing GAGs will increasingly play a crucial role in advancing our understanding of sepsis pathogenesis.

In summary, GAGs play a key role in the microvascular coagulation observed in sepsis. However, the molecular mechanisms underlying this phenomenon remain unclear and warrant further investigation. The utilization of sensitive and accurate quantitative techniques for GAGs will significantly contribute to elucidating the mechanism of microcoagulation in sepsis. Furthermore, these techniques can serve as dynamic monitoring methods for sepsis patients, enabling the efficient prediction and adjustment of clinical treatment strategies.

What is known about this topic?

- Coagulation dysfunction in sepsis is closely related to endothelial damage.
- Glycosaminoglycans, covering the surface of endothelial cells, have the potential to predict the state of endothelial cell injury.
- Little is known about the association between glycosaminoglycans and thrombosis.

What does this paper add?

- We summarized the potential pathways of glycosaminoglycans participating in sepsis-induced thrombosis.
- Generalizing the advanced detection methods of glycosaminoglycans and comparing different LC-MS methods.

Data Availability Statement

Not applicable. All the data supporting in this article can be found in publicly available datasets.

Authors' Contribution

All authors drafted the article and revised it critically for important intellectual content and approved the final manuscript to be published.

Competing Interests

None declared.

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