Circulating Heparan Sulfate Proteoglycans as Biomarkers in Health and Disease

Circulating Heparan Sulfate Proteoglycans as Biomarkers in Health and Disease

Antonio Junior Lepedda[Q1], PhD[1] Gabriele Nieddu, PhD[1] Zoi Piperigkou, PhD[2,3]
Konstantina Kyriakopoulou[2,3Q2] Nikolaos Karamanos, PhD[2,3] Marilena Formato[1]

[1] Department of Biomedical Sciences, University of Sassari, Sassari, Italy
[2] Biochemistry, Biochemical Analysis and Matrix Pathobiology Research Group, Laboratory of Biochemistry, Department of Chemistry, University of Patras, Patras, Greece
[3] Foundation for Research and Technology-Hellas (FORTH)/Institute of Chemical Engineering Sciences (ICE-HT), Patras, Greece

Address for correspondence Marilena Formato, [Q3]Department of Biomedical Sciences, University of Sassari, Viale San Pietro, 43/B, 07100, Sassari, Italy (e-mail: formato@uniss.it).

Semin Thromb Hemost 2021;00:1–36.

Abstract

Cell-surface heparan sulfate proteoglycans (HSPGs) play key roles in regulating cell behavior, cell signaling, and cell matrix interactions in both physiological and pathological conditions. Their soluble forms from glycocalyx shedding are not merely waste products, but, rather, bioactive molecules, detectable in serum, which may be useful as diagnostic and prognostic markers. In addition, as in the case of glypican-3 in hepatocellular carcinoma, they may be specifically expressed by pathological tissue, representing promising targets for immunotherapy. The primary goal of this comprehensive review is to critically survey the main findings of the clinical data from the last 20 years and provide readers with an overall picture of the diagnostic and prognostic value of circulating HSPGs. Moreover, issues related to the involvement of HSPGs in various pathologies, including cardiovascular disease, thrombosis, diabetes and obesity, kidney disease, cancer, trauma, sepsis, but also multiple sclerosis, preeclampsia, pathologies requiring surgery, pulmonary disease, and others will be discussed.

Keywords
- syndecans
- glypicans
- glycocalyx shedding
- circulating HSPGs
- disease biomarkers

Proteoglycans (PGs) are a ubiquitous family of macromolecules consisting of specific core proteins bearing one or more O- or N-linked sulfated glycosaminoglycan (GAG) chains, with key structural and functional roles in many tissues that can be found in both plasma and urine. GAGs are a group of complex anionic unbranched heteropolysaccharides composed of repeating disaccharide units containing an hexosamine, either N-acetylgalactosamine (GalNAc) or N-acetylglucosamine (GlcNAc), and an hexuronic acid (glucuronic acid [GlcA] or its carbon-5 epimer iduronic acid [IdoA]) or galactose (in keratan sulfate). GAGs are very heterogeneous polysaccharides in terms of the type of repeating disaccharide unit, chain length, charge density, degree and pattern of sulfation, degree of epimerization, and physicochemical properties, being responsible for most of the numerous biological functions of PGs.[1] Six main classes of GAGs have been described: hyaluronan or hyaluronic acid (the only free, nonsulfated GAG), chondroitin sulfate, dermatan sulfate, keratan sulfate, heparan sulfate (HS), and heparin. PGs can be found in the extracellular matrix (ECM), i.e., hyalectans, decorin, biglycan, and basement membrane PGs, associated with the cell surface, i.e., syndecans (SDCs) and glypicans (GPCs), or intracellularly, serglycin (the only currently known).[2]

In particular, heparan sulfate PGs (HSPGs) can exert an area of biological actions from regulation of cell–matrix interactions to cell signaling, not only when anchored in the cell surface but also in their soluble form after PG shedding. In recent years, accumulating evidence has

Issue Theme Hemostatic and Nonhemostatic Effects of Heparan Sulfate Proteoglycans; Guest Editors: Yona Nadir, MD, PhD and Ton Lisman, PhD.

Thieme Medical Publishers, Inc., 333 Seventh Avenue, 18th Floor, New York, NY 10001, USA

DOI https://doi.org/10.1055/s-0041-1725063.
ISSN 0094-6176.

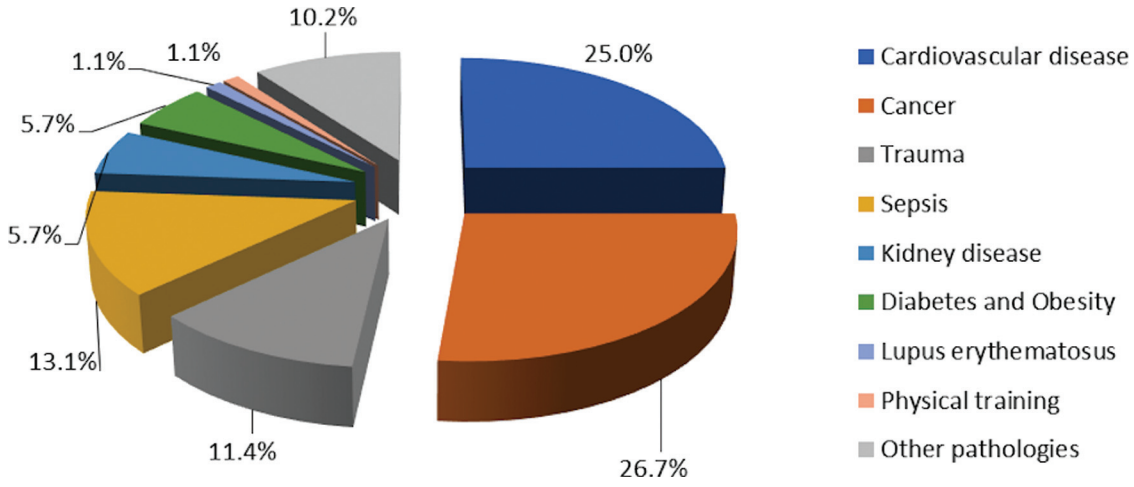

Fig. 1 Distribution[Q4] of the last 20 years' clinical studies in which circulating heparan sulfate/heparan sulfate proteoglycan levels have been used as markers of pathological conditions (https://pubmed.ncbi.nlm.nih.gov/).[Q5]

indicated that any alterations in HSPGs' expression, regulation, or functions could be key to overcome drug resistance and advance chemotherapeutic potency.

Herein, we performed an in-depth literature search using MEDLINE (PubMed) with the aim of collecting several authoritative reviews and original studies on the topic, published between January 2000 and April 2020. The following search words were used: extracellular matrix (ECM), circulating heparan sulfate (HS)/heparan sulfate proteoglycans (HSPGs), hyaluronic acid, glycosaminoglycans (GAGs), syndecans (SDCs), glypicans (GPCs), glycocalyx/shedding, and circulating biomarkers. About 400 articles were selected to be considered for this comprehensive review. Among them, a total of 176 clinical studies dealt with the association among circulating HS/HSPG levels, mainly from endothelial glycocalyx degradation, and several pathophysiological conditions including, most frequently, cardiovascular disease, cancer, trauma, sepsis, kidney disease, diabetes, and obesity, but also multiple sclerosis, preeclampsia, pathologies requiring surgery, pulmonary disease, and others, as shown in ►**Fig. 1**. Noteworthy, in almost all of them, the HSPGs SDCs, GPCs, or their ectodomains from the endothelial glycocalyx degradation were chosen as biomarkers.

The focus of this manuscript will be to review the last 20 years' clinical literature and provide an overview of the role and significance of circulating HSPGs in prognosis and diagnosis of various pathologies, the cases under study, and the main conclusions. Readers are referred to excellent reviews on topics such as ECM structure,[3] PG structure and function,[2] and endothelial glycocalyx structure and function[4,5] for more detailed discussion.

The Endothelial Glycocalyx and Its Remodeling

The luminal surface of vascular endothelial cells is lined by a dynamic and complex gel-like network, or glycocalyx, consisting of PGs (SDCs, GPCs), glycoproteins (selectins, integrins, and immunoglobulins), GAGs (hyaluronan, HS, and chondroitin sulfate), and soluble proteins from both plasma and endothelium, which represents an interface between endothelial cells and flowing blood (►**Fig. 2**).

This structure was visualized, for the first time, by Bennett et al, using electron microscopy,[6] who termed it "glycocalyx" (literally meaning "sugar coat" from the ancient Greek words glykys/γλυκύς = sweet, kylix/κύλιξ = cup) for the high content of polysaccharides of which the polyanionic acidic HS, chondroitin sulfate isomers, and hyaluronan represent major constituents. The thickness (~0.5–5.0 μm) and structure of the glycocalyx vary in relation to the species, vascular bed, organ, and blood flow rate[7] as well as with several pathophysiological conditions, and they are the result of a dynamic balance between constant biosynthesis of new glycans and shear-dependent alterations, with a considerable rate of turnover of its components. With regard to hyaluronan, this rate is 5 g per day, leading to a complete renewal of the entire pool every 3 days.[8] HS is covalently bound to core proteins to form the main endothelial glycocalyx PGs, SDCs, and GPCs (roughly 50–90% of the total amount of PGs), whereas hyaluronic acid binds noncovalently membrane proteins, such as CD44, providing structural support to the network. The endothelial glycocalyx represents a blood-vessel component fundamental to homeostasis with an extent of structural and functional roles, including controlling vascular permeability (by the highly negatively charged GAG constituents) and microvascular tone through mechanosensing (induction of endothelial nitric oxide synthesis by shear stress), preventing microvascular thrombosis, and regulating leukocyte adhesion (antiadhesive properties). In this respect, the endothelial glycocalyx retains and interacts with several enzymes, lipoproteins, and other proteins from plasma involved in oxidative and inflammatory pathways such as extracellular superoxide dismutase, xanthine oxidoreductase, low-density lipoproteins and lipoprotein lipase, interleukins (ILs), such as IL2–IL5, IL7, IL8, IL12, basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), and transforming growth factor β (TGF-β), as well as with regulators of hemostasis, such as antithrombin, heparin cofactor II, and tissue pathway factor inhibitor.[5] Derangement of the endothelial glycocalyx structure by either physical or chemical

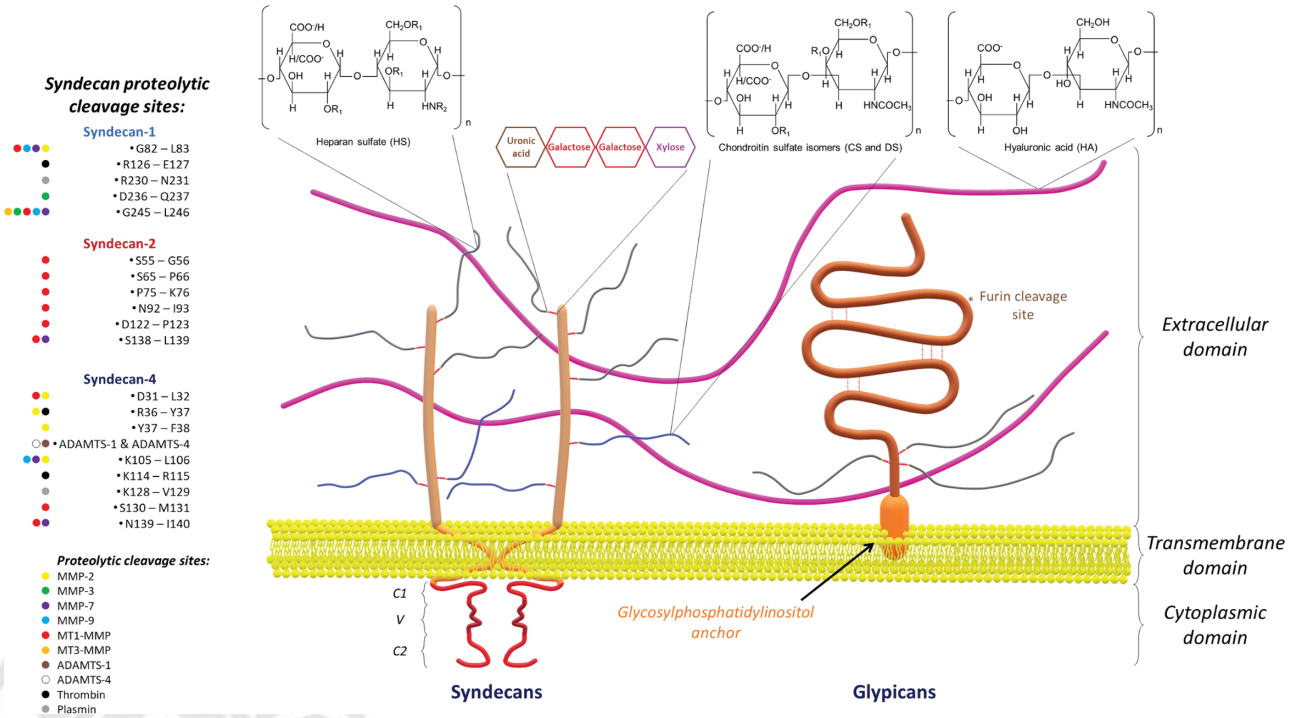

Fig. 2 Schematic representation of the endothelial glycocalyx and its main components showing some details of the syndecans, glypicans, and hyaluronic acid structures. Major proteolytic cleavage sites of syndecans for some metalloproteases (MMPs) and serine proteases are reported.[55] $R_1 = SO_3^-$; $R_2 = COCH_3/SO_3^-$. Amino acid residues are reported according to one-letter code.

insults plays major roles in several pathological conditions, such as cardiovascular disease,[9] diabetes,[10] kidney disease,[11] sepsis,[12,13] and trauma.[14] In particular, glycocalyx shedding,[15] a regulated proteolytic cleavage of some of its components, may expose, in pathological conditions, adhesion molecules, such as selectins and intercellular adhesion molecule 1, on the endothelium surface, thus inducing leukocyte and platelet recruitment leading to vascular dysfunction.[16,17] Furthermore, it increases plasma concentration of several glycocalyx components, such as hyaluronan, HS, and their degradation products, ectodomains of SDCs and GPCs, which may affect both local and systemic signaling pathways[18] and persist even for days, as demonstrated by highly sulfated HS fragments released in circulation by patients with respiratory failure.[19]

Under various acute and chronic clinical conditions, the glycocalyx shedding primarily involves various metalloproteases (MMPs), A disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-1 and -4 (►**Fig. 2**), heparanase and hyaluronidase released by inflammatory cells and activated endothelial cells, reactive oxygen species (ROS), and reactive nitrogen species (RNS). Serine proteases, such as thrombin, elastase, proteinase 3, plasminogen, and cathepsin B, are involved in glycocalyx shedding as well.[15]

MMPs (MT1-MMP, MMP-2, -7, and -9) and heparanase stored by endothelial cells are secreted following inflammatory stimuli, contributing to the cleavage of both the protein core and the HS moiety of SDCs and GPCs. Heparanase is an endoglycosidase that degrades HS, mainly at sites of low sulfation, releasing fragments of 4 to 7 kDa.[20] Activity of MMPs is modulated by tissue inhibitors of metalloproteinases (TIMPs), which can bind HS and chondroitin sulfate in the glycocalyx, whereas heparanase can be inhibited by heparin.[21] In this respect, Sulodexide, a commercial mixture of low-molecular-weight heparin (82%) and chondroitin sulfate isomers (18%), used as antithrombotic and profibrinolytic drug, shows anti-inflammatory, antioxidant, and vasculoprotective properties, probably by inhibiting heparanase and MMP-9 activity, thereby preserving endothelial glycocalyx.[22]

Hyaluronan, a high molecular weight GAG (up to 3–4 MDa) consisting of 1 to 2 µm unbranched free chains, is a key player in maintaining endothelial glycocalyx integrity and another target during its degradation. Hyaluronan is a nonsulfated GAG consisting of repeating residues of GlcNAc and GlcA connected by β-1,3- and β-1,4-glycosidic bonds.[23] In contrast to the other GAGs, which are synthesized in the Golgi apparatus covalently linked to protein cores, hyaluronan is synthesized by membrane-bound synthases at the inner side of the plasma membrane and extruded through pore-like structures to the cell surface without any further postsynthetic modifications (i.e., sulfation or epimerization).[24] Hyaluronan weaves into the endothelial glycocalyx through its interaction with surface hyaluronan receptors, such as the transmembrane CD44, and chondroitin sulfate chains.[4]

During glycocalyx shedding, its degradation occurs by the action of hyaluronidase 1, the most active somatic hyaluronidase that is thought to be enriched in endothelial cells through endocytosis from the bloodstream. Also, hyaluronidase 2, a glycosylphosphatidylinositol (GPI)-anchored enzyme located at the surface of the endothelial cells, contributes to hyaluronan fragmentation. Their action leads to the formation of low molecular weight (<500 kDa) fragments that, conversely, are proinflammatory through induction of the expression of

chemokines and metalloproteinases, so contributing to endothelial derangement.[25,26] Together with the constantly changing physical forces from the bloodstream (the pulsatile blood flow and passage of formed elements) acting on the surface of endothelial cells, these mechanisms may induce endothelial dysfunction.

ROS and RNS can lead to glycocalyx fragmentation both directly via GAG fragmentation[27,28] and, indirectly, by activating MMPs and inactivating their endogenous inhibitors (TIMPs).[29]

Moreover, the sialic acid residues on glycoproteins and glycosphingolipids of the endothelial glycocalyx seem to play a role in both scavenging hydrogen peroxide[30] and regulating microvessel permeability,[31] suggesting that desialylation may represent a further mechanism of glycocalyx derangement.

As mentioned above, several diseases characterized by systemic vascular inflammation show endothelial glycocalyx degradation with the consequent release of some of its components, such as soluble SDC-1, HS, and hyaluronan, into the blood stream. Conversely, it has been shown that physical exercise may preserve endothelial glycocalyx integrity by reducing oxidative stress.[32,33] Serum levels of these fragments are a direct index of glycocalyx damage and, in some cases, have been demonstrated as useful markers of disease severity (►**Supplementary Tables S1**–S5,[Q6] available online only).

Cell-Surface Proteoglycans

The cell-surface PGs include two major subfamilies: the type I transmembrane SDCs and the glycosylphosphatidylinositol-anchored GPCs. They are not merely structural glycocalyx components but play major roles in regulating cell behavior, cell signaling, and cell matrix interactions.

SDCs are a conserved family of single-span transmembrane PGs carrying HS chains (both SDC-1 and -3 bear also two chondroitin sulfate chains). They play pivotal roles in development, inflammation, and tumor progression by controlling cell proliferation, differentiation, adhesion, and migration.[34,35] In vertebrates, four SDC genes code for SDC-1, -2, -3, and -4, with distinct temporal and spatial pattern of expression. SDC-1 is widely expressed in epithelial and plasma cells, SDC-2 is mainly expressed in mesenchymal cells, such as fibroblasts and smooth muscle cells, SDC-3 is expressed in neural tissues and developing musculoskeletal tissues, whereas SDC-4 is abundant in most tissues.[34] As they are involved in several signaling events, including tumorigenesis, their expression is finely regulated. Their protein cores range from 20 to 40 kDa (33, 41, 23, and 22 KDa, respectively, for SDC-1, -2, -3, and -4), each containing a short C-terminal cytoplasmic domain, a transmembrane domain, and a ectodomain that carry three to five HS or chondroitin sulfate chains (►**Fig. 2**). Although only few structural data are available probably due to the high percentages of disordered amino acid residues, a model of full-length SDC-3 has been generated.[36] SDC-4 exists also as a soluble protein isoform produced by alternative splicing removal of the transmembrane domain.[37] The short cytoplasmic domain interacts with several intracellular kinases, as well as with actin cytoskeleton, playing a crucial role in modulating various cell functions.[38] It consists of two conserved regions, one near the transmembrane domain and the other at the C-terminal (C1 and C2, respectively), and of a variable region between them (►**Fig. 2**). The C1 domain, which is identical in all four mammalian SDCs, is involved in SDC di- or oligomerization and in binding of several intracellular proteins. In SDC-4 this region includes a phosphatidylinositol-4,5-bisphosphate (PIP_2) binding site that is involved in its dimerization and is required for protein kinase C activation and intracellular signaling.[39–41] The variable domain, specific to each SDC, is highly conserved across species. The C2 domain is likely to be involved in intracellular transduction by organizing signaling complexes at the cell membrane. In particular, it contains a conserved carboxyl-terminal EFYA tetrapeptide sequence that binds some PDZ domain-containing proteins that may function as scaffold proteins that recruit signaling and cytoskeletal proteins to the plasma membrane.[42] The transmembrane domain is highly conserved and contains a small sequence (a GXXXG motif) that promotes self-association leading to dimer/oligomer formation required for protein kinase C activation.[43] The ectodomain, interacting with the extracellular milieu primarily by its GAG chains, is fundamental to the mechanisms of signal transduction.[44] Its shedding, mainly by metalloproteinases, occurs constitutively at specific sites,[15] but is rather accelerated in response to inflammatory stimuli and pathological conditions.[5,45,46] This allows for a rapid modulation of intracellular response by reducing surface receptors as well as generating soluble bioactive ectodomains. HS chains are covalently O-linked to serine residues in a serine–glycine motif surrounded by acidic residues near the N-terminal of ectodomain. Furthermore, SDC-1 and -3 carry chondroitin sulfate/dermatan sulfate chains at sites closer to the transmembrane domain. HS and chondroitin sulfate/dermatan sulfate chains are polymerized in the Golgi apparatus, starting from a tetrasaccharide linker consisting of xylose–galactose–galactose–uronic acid residues, by the sequential and repetitive addition of GlcA, and GlcNAc (HS chain) or GalNAc (chondroitin sulfate chain) residues to reach 50 to 200 disaccharides in length. Both epimerization of GlcA to IdoA and sulfation at specific positions occur during polymerization, leading to extensive postsynthetic chain modification. Regarding HS, hexuronic acid residues may be sulfated at the 2-O position, whereas N-glucosamine residues may become sulfated at the N-position, the 6-O position, or, less frequently, at the 3-O position, by specific sulfotransferases. The different disaccharide species are not randomly distributed but produce alternating subdomains of low (N-acetylated domains) and high sulfation (N-sulfated domains) with intermediate sulfation sequences in between (N-sulfated/N-acetylated domains) (►**Fig. 3**).

Once they have reached the cell surface, HS chains may be further modified by selective removal of 6-O sulfates by specific sulfatases.[47–49] Their potential structural heterogeneity, in terms of chain length, degree, and pattern of iduronation and sulfation, which is further increased by dynamic modifications in response to cellular and environmental stimuli, is so substantial that, virtually, there are not two GAGs identical in the body. HS chains interact with a wide range of ligands such as ECM proteins (e.g., collagens, fibronectin), plasma proteins (e.g., albumin, antithrombin), cytokines, chemokines, growth

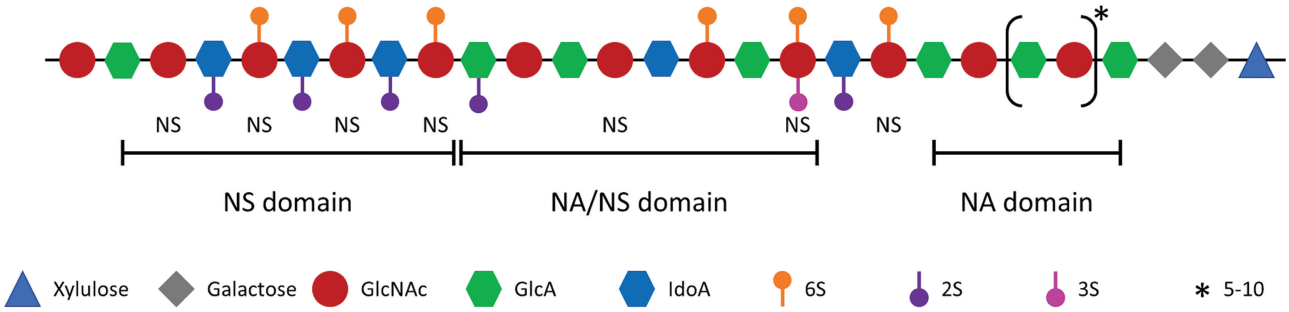

Fig. 3 The repetitive domain organization of heparan sulfate in which highly sulfated/iduronated regions (N-sulfated domains or NS domains) are flanked by moderately modified regions (N-acetylated/N-sulfated domains or NA/NS domains) with interspersed unmodified sequences (N-acetylated domains or NA domains).[141]

factors (e.g., FGF, VEGF, TGF-β, and platelet-derived growth factor [PDGF]), and enzymes, and are primarily involved in signal transduction by forming ternary complexes such as FGF2-FGFR1, with both growth factors and their receptors.[50,51] During glycocalyx shedding, HS chains may be cleaved at the low-sulfated domains by the endo-β-D-glucuronidase heparanase, which releases oligosaccharides of 10 to 20 sugar residues, thereby turning HS from an inhibitor to a potent activator of FGF-2.[52,53] Although the biological function of the SDC chondroitin sulfate chains is not completely understood, it has been suggested that it may cooperate with HS chains to modulate cell adhesion to the ECM.[54] Very recently, Gondelaud and Ricard-Blum provided a comprehensive SDC interactome, showing an impressive number of partners (351), including those identified by the high-throughput method affinity purification-mass spectrometry.[55] Very interestingly, the vast majority of SDC partners was shown to interact with their GAG chains (71% for SDC-1, 67% for SDC-2, 91% for SDC-3, and 68% for SDC-4).

GPCs are a family of six (in mammals) GPI-anchored HSPGs (GPC-1 to -6), organized into two subfamilies (GPCs -1, -2, -4, -6 and GPCs -3 and -5) with approximately 25% amino-acid identity between groups. Fourteen well-conserved cysteine residues, forming seven disulfide bridges in a large globular cysteine-rich domain, stabilize very similar three-dimensional structures.[56,57] They have core proteins ranging from 60 to 70 KDa, and an hydrophobic C-terminal domain required for the binding of the GPI anchor, which links GPCs to specific membrane domains (lipid rafts), enriched in cholesterol- and sphingolipids, involved in vesicular transport and cell signaling.[58] Attachment sites for three to four HS chains are limited to the last 50 amino acids in the C-terminus, close to the membrane. During maturation in the secretory pathway, they can be cleaved by the endoprotease furin (between Arg^{358} and Cys^{359} in GPC-3) that generates two fragments (a 40-kDa N-terminal subunit and a 30-kDa C-terminal subunit) connected by disulfide bridges.[59] Several forms of GPCs can be found also in circulation, such as full-length glycated forms released by Notum, a kind of lipase that cleaves GPI-anchored proteins,[60] or N-terminal fragments lacking HS side chains.[61] In general, GPCs are highly expressed during embryonic development with tissue-specific and stage-specific expression, suggesting a role in fundamental biological processes such as cell–ECM interactions and the control of cell division, differentiation, and morphogenesis.[56] GPC-1 is expressed in bone, bone marrow, muscle, epithelium, and kidney; GPC-2 is specifically expressed in the nervous system; GPC-3 and GPC-6 are expressed in most tissues; GPC-4 is expressed in brain, kidney, and lung; GPC-5 is expressed in brain, lung, liver, kidney, and limbs. In adults, the pattern of GPC expression changes dramatically. In particular, GPC-1, GPC-4, and GPC-6 are widely expressed in various tissues; GPC-2 is no longer expressed; GPC-3 is only expressed in the ovary, mammary gland, mesothelium, lung, and kidney; and GPC-5 is specifically expressed in the brain.[62] GPC-1 is the only one expressed by endothelial cells.[63] GPCs are involved in the regulation of pathways including Wnt, FGF, hedgehog (Hh), bone morphogenic protein, slit, and insulin-like growth factor, with either stimulatory or inhibitory activity depending on the biological context.[2,57]

Circulating HSPGs as Disease Biomarkers

As mentioned above, cell-surface PGs and their degradation products may be released into circulation by several mechanisms, such as shedding by the action of MMPs and other enzymes, alternative splicing, and secretion of soluble isoforms. They may represent potential markers of several pathophysiological conditions useful for diagnostic, prognostic, or therapeutic purposes. Their high levels in blood may be associated to an exacerbation of the above-mentioned mechanisms on constitutively expressed PGs (e.g., those present in the glycocalyx) or to a de novo expression in a pathological condition (e.g., the expression of the oncofetal GPC-3 by hepatocellular carcinoma).

In this manuscript we have collected many studies assessing the clinical utility of measuring circulating HS/HSPGs in several pathophysiological conditions (see the Supplementary tables, , available online only). As shown in ►**Fig. 1**, most of them focused on cardiovascular disease and related disorders, including diabetes, obesity, and kidney disease (~36% of the included studies; ►**Supplementary Table S1**, available online only), cancer (28%; ►**Supplementary Table S2**, available online only), trauma (11%; ►**Supplementary Table S3**), and sepsis (13%; ►**Supplementary Table S4**, available online only), but also multiple sclerosis, preeclampsia, pathologies requiring surgery, pulmonary disease, and others (►**Supplementary Table S5**, available online only). The main findings on both cardiovascular disease and cancer will be discussed below.

Readers are referred to the studies of Tuma et al[14] and Uchimido et al,[13] for detailed reviews on the association between glycocalyx degradation and trauma or sepsis, respectively.

Cardiovascular Disease and Related Disorders

Cardiovascular disease is the leading cause of morbidity and mortality in the world, being heart disease, mostly due to atherosclerosis of the coronary arteries, and stroke, the underlying cause of 31% global deaths.[64] Originally thought merely as an inevitable degenerative consequence of aging, atherosclerosis is rather a chronic systemic inflammatory condition with a very complex etiology in which hypertension, diabetes, obesity, and hyperlipidemia play key roles. It is characterized by the accumulation of lipids and fibrous elements into the subendothelial space of arteries leading to plaque formation, which ultimately could evolve into an acute clinical event due to plaque rupture and thrombosis.[65]

Oxidative stress has been long associated with arterial dysfunction contributing to atherogenesis and, ultimately, to plaque development and degeneration toward prone-to-rupture lesions. In this respect, in situ oxidative events, mainly caused by ROS, may have important functional consequences if not properly counteracted by the pool of enzymes and nonproteinaceous antioxidants of the arterial wall.[66] An intact glycocalyx, with its superoxide dismutase, represents a key functional barrier to plasma oxidants, protecting the vascular endothelium from reduced nitric oxide bioavailability, due to both impaired endothelial nitric oxide synthase (eNOS) activity and NO oxidation by superoxide anion, forming peroxynitrite anion, a RNS. As mentioned above, besides ROS and RNS, sheddases, mainly matrix metalloproteinases, heparanase, and sialydases, can damage the glycocalyx, leading to increased permeability, leucocyte–endothelium interactions, thrombosis, and vascular inflammation, in a vicious cycle. Evidence suggests that a damaged glycocalyx, found in aging, may initiate endothelial dysfunction leading to cardiovascular disease.[9] Accordingly, in the last 20 years, a plethora of studies have shown the association between circulating HSPG levels, mainly SDC-1 and -4, and cardiovascular disease, including myocardial infarction,[67–70] heart failure,[71–74] stroke,[75] and coronary artery disease[76] (see ►**Supplementary Table S1**, available online only).

Diabetes mellitus is a huge global health problem, affecting more than 463 million people worldwide, and a major risk factor for cardiovascular diseases, such as coronary artery disease, stroke, and peripheral vascular disease (International Diabetes Federation, ATLAS 2019, https://www.diabetesatlas.org/en/).[77,78] Type 1 diabetes mellitus results from the autoimmune destruction of the insulin-producing β cells of Langerhans islets; it is usually diagnosed in children and young adults and represents less than 10% of all cases of diabetes. Type 2 diabetes mellitus, or adult-onset diabetes, represents over 90% of cases of diabetes mellitus and is characterized by hyperglycemia caused by insulin resistance.

Hyperglycemia results in a profound systemic derangement of the endothelial glycocalyx, with a concomitant increase in vascular permeability and flow-dependent arterial dilatation, which contributes to the increased risk of macrovascular and microvascular complications in diabetic patients.[79,80] In this respect, high serum levels of soluble SDC-1 have been reported for both type 1[81,82] and type 2[83] diabetic patients, also in relation to kidney function. In particular, chronic kidney disease represents a frequent diabetes complication that may lead to kidney failure, hemodialysis, and, ultimately, to renal transplantation. All these conditions have been associated with systemic glycocalyx degradation and increased levels of soluble SDC-1.[84–88] In kidney, derangement of the glomerular basement membrane glycocalyx, which is an early event in diabetic nephropathy, involves the loss of negatively charged functional groups, especially HS and HSPGs, with a consequent reduction of the charge-dependent glomerular permselectivity. High urinary excretion of soluble HSPGs, HS, chondroitin sulfate as well as of bikunin (a small circulating chondroitin sulfate PG also known as urinary trypsin inhibitor), which has been suggested to precede microalbuminuria, has been long associated with the early events of renal derangement in diabetes.[89]

Obesity is another major continuously growing health care problem. Associated with type 2 diabetes mellitus, dyslipidemia, and hypertension, it has devastating effects on vascular function favoring cardiovascular disease onset. Adipose tissue is not simply a fat storage tissue, rather, it functions as an endocrine organ by secreting multiple bioactive proteins involved in both inflammation and insulin metabolism, known as adipokines. Obesity is associated with increased expression of proinflammatory adipokines resulting in the development of a chronic, low-grade inflammatory state and insulin resistance.[90]

In 2006, Gesta et al showed that GPC-4 was more expressed in subcutaneous adipose tissue than in visceral adipose tissue and that it strongly correlated with body mass index (BMI) and waist-to-hip ratio in a tissue-specific way, positively in the former and negatively in the latter.[91] In 2012, the same research team demonstrated that GPC-4 was released by adipose cells and that its serum levels correlated with BMI and insulin sensitivity. Based on these studies, they suggested GPC-4 as a novel circulating insulin sensitizing adipose-derived factor, which may interact directly with the insulin receptor, enhancing insulin signaling and adipocyte differentiation.[92]

In an attempt to clarify the relationships among individual adipokines and between adipokines and obesity, glucose metabolism, and inflammation, in 2014, Flehmig et al assessed serum concentrations of 20 adipokines in 141 Caucasian obese subjects within a wide range of body weight, glycemia, and insulin sensitivity. Following distance-based hierarchical cluster analyses, they identified two major adipokine clusters associated with either body fat mass and inflammation or insulin sensitivity/hyperglycemia, and lipid metabolism, belonging GPC-4 to the latter.[93]

The association between GPC-4, glucose metabolism, and obesity was further investigated in two cross-sectional studies. The first one, performed on 170 obese patients grouped

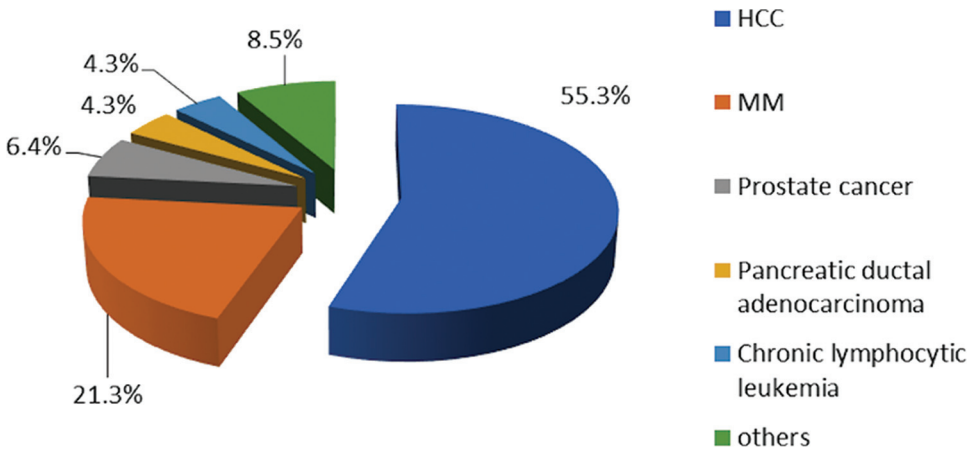

Fig. 4 Distribution of the last 20 years' clinical studies in which circulating heparan sulfate/heparan sulfate proteoglycans levels have been used as a marker in cancer (https://pubmed.ncbi.nlm.nih.gov/). HCC, hepatocellular carcinoma; MM, multiple myeloma.

according to their different glucose metabolism status and 38 normal controls, showed that serum GPC-4 levels were significantly elevated in obese patients with insulin resistance and correlated positively with BMI, systolic blood pressure, alanine aminotransferase, aspartate aminotransferase, fasting insulin, and homeostasis model assessment of insulin resistance.[94] The second one assessed GPC-4 levels in 370 children, aged 6 to 18 years, showing a positive correlation with BMI, lipid profile, aspartate aminotransferase, and alanine aminotransferase, whereas no associations with insulin sensitivity and β-cell function indices were demonstrated.[95]

Cancer

As shown in ►**Fig. 4**, the vast majority of the selected studies have been focused on two cancer types responsible for immense health care problems, hepatocellular carcinoma and multiple myeloma, which will be discussed below. With regard to other cancer types, readers are referred to ►**Table 1** and ►**Supplementary Table S2**, available online only, for the main findings.

Hepatocellular Carcinoma

According to the International Agency for Research on Cancer, liver cancer is the third most common cause of cancer death leading to 781,631 deaths worldwide (8.2%; source: Globocan 2018), with hepatocellular carcinoma being the major contributor. Hepatocellular carcinoma is associated primarily with chronic persistent infection of hepatitis B or C virus but also with risk factors such as alcohol consumption or chemical and aflatoxin B1 exposure. Due to its aggressiveness, it is associated with a poor prognosis and considerably low 5-year survival rates.[96]

In 1997, Hsu et al first reported that GPC-3 mRNA was highly expressed in placenta, fetal liver, lung, and kidney, but it was undetectable in adult liver and barely detectable in adult lung and kidney. Furthermore, it was highly and preferentially expressed in hepatocellular carcinoma.[97] Since these findings, to date many studies have shown that GPC-3 is a reliable immunohistochemical diagnostic marker of hepatocellular carcinoma, associated with a poor prognosis. In this respect, a significant association among GPC-3 and tumor stage, tumor differentiation, presence of vascular invasion, and metastasis has been demonstrated (►**Table 2**).[98–102] Being highly and specifically expressed on the cell surface of hepatocellular carcinoma cells but barely in the other adult cells, it is becoming also a promising target for immunotherapies, which involve the use of humanized anti-GPC-3 cytotoxic antibodies, treatment with peptide/DNA vaccines, immunotoxin, or genetic therapies.[103,104]

As mentioned above, during maturation, GPC-3 can be cleaved by the endoprotease furin between Arg^{358} and Cys^{359} residues, generating a 40-kDa NH_2-terminal fragment and a 30-kDa GPI-anchored fragment, linked together by disulfide bonds.[59] The former can be found in plasma of hepatocellular carcinoma patients representing a serological marker for early-stage hepatocellular carcinoma.[61]

In the last 20 years, several studies have been performed, with the aim of validating serum GPC-3 as a noninvasive diagnostic marker of hepatocellular carcinoma, sometimes with conflicting results (see ►**Supplementary Table S2**, available online only), most of which have been included in the five meta-analysis studies reported in ►**Table 3**.[105–109] These studies calculated pooled accuracy parameters showing quite similar sensitivities and specificities and high diagnostic odds ratios suggesting serum GPC-3 as a promising diagnostic marker for hepatocellular carcinoma. However, there was great heterogeneity among studies (I^2 values considerably higher than the cut-off value of 50%, reported in ►**Table 3**) and major design deficiencies such as differential verification bias, and a lack of clear exclusion and inclusion criteria leading to nondefinitive conclusions.

Recently, a few studies have been published searching for methods to improve the diagnostic power of GPC-3 for hepatocellular carcinoma (see ►**Supplementary Table S2**, available online only). Among them, Attallah et al developed a GPC-hepatocellular carcinoma model based on combination of GPC-3 levels and routine laboratory tests including alanine transaminase, aspartate transaminase, alkaline phosphatase, total bilirubin, albumin, platelet count, GPC-3, and α-fetoprotein, showing high hepatocellular carcinoma diagnostic power

Table 1 Main findings of studies correlating circulating HSPG levels with tumors other than hepatocellular carcinoma or multiple myeloma[Q7Q8]

References	Heparan sulfate proteoglycan	Tumor typology	Casuistry	Main findings
130	SDC-1	Hodgkin's lymphoma	66 patients vs. 14 healthy controls	100.2 ± 35.9 vs. 67.9 ± 24.5 ng/mL ($p < 0.001$)
131	Perlecan	Prostate cancer	288 prostate cancer patients vs. 12 healthy controls	Perlecan fragments associated with MMP-7 in prostate cancer tissues; domain IV perlecan in stage IV, but absent in normal sera. Perlecan fragments in sera and MMP-7 in tissues are measures of invasive prostate cancer
132	GPC-1	Prostate cancer	15 prostate cancer patients vs. 15 benign prostatic hyperplasia vs. controls	GPC-1 reduction in prostate cancer patients ($p < 0.05$)
133	SDC-1	Castration-resistant prostate cancer (CRPC)	75 patients who received docetaxel therapy until the appearance of therapy resistance	Serum SDC-1 may help to facilitate clinical decision-making regarding the type and timing of therapy for patients with CRPC; positive correlation between SDC-1 and MMP7
134	Exosomal GPC-1	Pancreatic ductal adenocarcinoma (PDAC)	3 PDAC patients vs. 3 chronic pancreatitis vs. 6 healthy controls	GPC-1 is not diagnostic of PDAC
135	Exosomal GPC-1	Breast cancer, PDAC	32 breast cancer vs. 190 PDAC vs. 100 healthy controls	Levels of GPC1+ circulating exosomes correlate with tumor burden and the survival of pre- and postsurgical patients
136	SDC-1	Lung cancer	184 patients (138 non-small cell lung cancer and 46 small cell lung cancer)	Survival: SDC-1 > 59 ng/mL vs. SDC-1 < 59 ng/mL, 4 months vs. 11 months ($p < 0.0001$)
137	SDC-1	Chronic lymphocytic leukemia	52 patients vs. 12 healthy controls	149.0 ng/mL (13.2–257.1 ng/mL) vs. 36.7 ng/mL (17.4–135.8 ng/mL)
138	SDC-1	Chronic lymphocytic leukemia	104 patients vs. 32 controls	52.8 ng/mL (13.4–252.7 ng/mL) vs. 19.86 ng/mL (14.49–33.14 ng/mL) ($p < 0.01$)
139	Exosomal GPC-1	Colorectal cancer (CRC)	102 CRC patients vs. 80 healthy controls	Plasma GPC-1+ exosomes, miR-96–5p, and miR-149 are specific markers for the diagnosis of CRC and targets for the therapy
140	GPC-3	Hepatoblastoma (HB)	134 HB patients vs. 30 patients with benign hepatobiliary disorders vs. 20 controls	1.93 ng/mL (0–31.19) vs. 1.74 - ng/mL (0–25.95) ($p = 0.6$) vs. 0.59 - ng/mL (0–6.20) ($p = 0.003$); GPC3 is inferior to AFP as a serum marker for HB

Abbreviation: AFP, alpha fetoprotein.

Table 2 Meta-analysis studies on the prognostic significance of high hepatic tissue glypican-3 expression in predicting overall survival and disease-free survival of hepatocellular carcinoma patients

References	Casuistry	Assay	Overall survival[a]	Disease-free survival[b]
98	493 patients/5 studies	IHC	HR = 2.18, 95% CI: 1.47–3.24, $p = 0.0001$	HR = 2.05, 95% CI: 1.43–2.93, $p < 0.0001$
99	1070 patients/8 studies	IHC	HR = 1.96, 95% CI: 1.51–2.55, $p = 0.000$	HR = 1.99, 95% CI: 1.57–2.51, $p = 0.000$
100	2336 patients/15 studies	IHC	HR = 1.38, 95% CI: 1.05–1.80, $p = 0.02$	HR = 1.98, 95% CI: 1.08–3.62, $p = 0.027$
101	2364 patients/14 studies	IHC	HR = 1.40, 95% CI: 1.07–1.85, $p = 0.02$	HR = 1.61, 95% CI: 1.13–2.30, $p = 0.008$
102	2618 patients/17 studies	IHC	HR = 1.57, 95% CI: 1.18–2.10, $p = 0.002$	HR = 1.93, 95% CI: 1.09–3.43, $p = 0.02$

Abbreviations: CI, confidence interval; HR, hazard ratio; IHC, immunohistochemistry.
[a]Association between overexpression of glypican-3 and decreased overall survival.
[b]Association between overexpression of glypican-3 and decreased disease-free survival.

Table 3 Meta-analysis studies on the diagnostic significance of high serum glypican-3 levels in hepatocellular carcinoma (HCC) patients

References	Casuistry	Assay	Sensitivity	Specificity	Positive likelihood ratio	Negative likelihood ratio	Diagnostic odds ratio (DOR)
[105]	898 HCC patients/ 12 studies	ELISA/radioimmunology	0.53 (95% CI: 0.49–0.57; I^2: 79.9%)	0.77 (95% CI: 0.74–0.81; I^2: 95.9%)	NR (I^2: 91.5%)	NR (I^2: 86.1%)	10 (95% CI: 2–38; I^2: 90.6%)
[106]	NR/18 studies	ELISA/chemiluminescence immunoassay	0.69 (95% CI: 0.55–0.80; I^2: 90.71%)	0.93 (95% CI: 0.85–0.97; I^2: 97.30%)	10.50	0.34	31 (95% CI: 11–92)
[107]	1,935 HCC patients/ 19 studies	ELISA/radioimmunology	0.55 (95% CI: 0.53–0.57)	0.84 (CI: 0.82–0.86)	5.22 (CI: 3.09–8.84)	0.54 (CI: 0.46–0.63)	13.8 (95% CI: 6.6–28.8)
[108]	1,201 HCC patients/ 17 studies	ELISA/chemiluminescence immunoassay	0.56 (95% CI: 0.53–0.59; I^2: 90.7%)	0.89 (95% CI: 0.87–0.90; I^2:94.3%)	7.82 (CI: 3.86–15.85; I^2: 93.9%)	0.48 (CI: 0.39–0.59; I^2: 85.9%)	26.73 (95% CI: 10.31–69.26; I^2: 90.0%)
[109]	11 studies	ELISA	0.55 (95% CI: 0.52–0.58; I^2: 93.3%)	0.58 (95% CI: 0.54–0.61; I^2: 90.2%)	1.69 (95% CI: 1.20–2.39)	0.67 (95% CI: 0.50–0.90)	3.64 (95% CI: 1.74–7.60; I^2: 85.3%)

Abbreviations: CI, confidence interval; ELISA, enzyme-linked-immunosorbent assay; HCC, hepatocellular carcinoma; NR, not reported.
Note: Positive likelihood ratio (PLR): true positive/false positive; negative likelihood ratio (NLR): false negative/true negative; diagnostic odds ratio (DOR): the odds of positive test results in patients with hepatocellular carcinoma/the odds of positive test results in patients without hepatocellular carcinoma; I^2: a quantitative measurement of inconsistency across different studies. I^2 value typically ranges from 0 (no observed heterogeneity) to 100% (maximal heterogeneity), and an I^2 value $\geq$ 50% is considered to represent substantial heterogeneity.

with a sensitivity of 93%, a specificity of 93%, a positive predictive value of 89%, a negative predictive value of 95%, and an efficiency of 93%, assessed on a cohort of 138 hepatocellular carcinoma patients, of whom 56 patients had cirrhosis, and 62 patients had liver fibrosis.[110] In 2017, Ofuji et al analyzed the association between perioperative serum GPC-3 levels and tumor recurrence rate in 25 patients with stage I hepatocellular carcinoma who underwent surgical resection, suggesting that they may be accurate predictors for recurrence after surgical resection of early-stage hepatocellular carcinoma.[111] Very recently, Shimizu et al showed a positive correlation among serum GPC-3 levels, its expression in hepatocellular carcinoma, and the comorbidity with hepatitis C virus infection in 56 patients with hepatocellular carcinoma, suggesting the utility of serum GPC-3 (as predictive of tissue GPC-3) in choosing the most appropriate therapy (e.g., immunotherapies targeting GPC-3).[112]

Multiple Myeloma

Multiple myeloma is a hematological malignancy characterized by uncontrolled expansion of malignant plasma cells in the bone marrow where they disrupt normal hematopoiesis and bone physiology. It may occur de novo or evolve from benign monoclonal gammopathy, spreading throughout the skeletal system, ultimately leading to death of almost all patients. In fact, although major progresses in treatment have been reached, multiple myeloma is still incurable, with a median survival of 4 to 6 years, mainly due to the ability of myeloma cells to develop drug resistance. It is characterized by a strong interaction between multiple myeloma cells and the bone marrow microenvironment that supports their survival and growth through signals mediated by adhesion molecules, cytokines, and growth factors.[113]

It is well known that SDC-1 is involved in multiple cellular processes, including proliferation, migration, adhesion, and angiogenesis. In this respect, it acts as a coreceptor for various heparin-binding growth factors, such as bFGF/FGF2, VEGF, TGF-β, and PDGF, and as a receptor, primarily through its HS chains. Dysregulation of its expression contributes to cancer progression. Generally, a lower expression in carcinoma cells contributes toward reduced cell adhesion to the ECM thus enhancing cell motility and invasion, whereas in some cases its higher expression in stroma alters the ECM organization contributing to angiogenesis and cancer progression.[114] In addition, shedding of its ectodomain by some matrix metalloproteinases/heparanase suggests activation of mechanisms promoting stemness and tumorigenesis.[115,116] Indeed, the soluble fragment that results after SDC-1 shedding is still bioactive and able to modulate the binding between growth factors and their receptors in tumor stroma or act as a reservoir of effector molecules, such as growth factors and chemokines, to promote endothelial cell invasion. It may also aid in cancer progression by sequestering inhibitory molecules.[114]

SDC-1 has a key role in normal B cell maturation, differentiation, and function and its expression is tightly regulated.[117] Furthermore, it is essential for the survival of long-lived plasma cells and multiple myeloma plasma cells.[118] SDC-1 is highly expressed by myeloma cells, and its ectodomain is

released in plasma by shedding. In this respect, heparanase, an endoglycosidase that releases bioactive fragments of 5 to 10 disaccharides from HS, plays a key role. In fact, its higher expression, with respect to normal tissues, strongly correlates with poor prognosis[116,119–121] and, therefore, it represents a potential target for treatment. In this respect, novel heparanase inhibitors, such as SST0001, have been developed to prevent degradation of HS and shedding of SDC-1, which are promising candidates for sensitizing myeloma cells to drug-induced growth inhibition.[122]

By the action of sheddases, soluble SDC-1 levels, which are usually low in healthy people, have been shown to increase significantly in plasma of patients with multiple myeloma (see ►**Supplementary Table S2**, available online only), representing a strong independent prognostic factor.[123,124] Indeed, higher plasma levels have been associated with both poor survival and resistance to chemotherapies.[125–127]

Overall, these studies show that soluble SDC-1 could be of great value for diagnosis, prognosis, and treatment response of multiple myeloma. Nonetheless, as SDC-1 is a key player in the crosstalk between multiple myeloma cells and bone marrow microenvironment, together with the machinery of HS synthesis and modification, it represents also a promising molecular target for therapeutic strategies.[117,122,128,129]

Conclusions

ECM is a three-dimensional complex macromolecular network that plays a crucial role in cell–cell communication and cell functions and contributes greatly in homeostasis. HSPGs are major components of ECM and their actions affect many critical cell processes including but not limited to cell invasion, migration, and adhesion. This in-depth review analyzes the key roles of cell-surface PGs in several threatening pathological conditions, with special focus on SDCs and GPCs, and expands on the relevance of their soluble forms for diagnostic, prognostic, as well as therapeutic purposes. However, among the large number of published studies, data are, sometimes, conflicting or of low clinical relevance. In this respect, the absence of common experimental design, including standard protocols for enrollment, and of robust diagnostics kits represent confounding issues that have to be solved.

Conflict of Interest

None declared.

Acknowledgments

A.J.L. thanks Regione Autonoma della Sardegna for its financial support (POR-FSE 2014–2020-Asse Prioritario 3 "Istruzione e Formazione"-Obiettivo Tematico: 10, Priorità d'investimento: 10ii, Obiettivo Specifico: 10.5, Azione dell'Accordo di Partenariato 10.5.12-C.U.P. J86C18000270002). G.N. thanks "Programma Operativo Nazionale (PON) Ricerca e Innovazione 2014-2020–Asse I "Capitale Umano," Azione I.2–A.I.M. Attraction and International Mobility, Linea 1 Mobilità dei ricercatori, AIM1874325–3, CUP: J54I18000110001" for its financial support. A.J.L., G.N., and M.F. thank the University of Sassari for its financial support (Fondo di Ateneo per la Ricerca 2019 and Fondo di Ateneo per la Ricerca 2020).

References

1 Karamanos NK, Piperigkou Z, Theocharis AD, et al. Proteoglycan chemical diversity drives multifunctional cell regulation and therapeutics. Chem Rev 2018;118(18):9152–9232
2 Theocharis AD, Skandalis SS, Tzanakakis GN, Karamanos NK. Proteoglycans in health and disease: novel roles for proteoglycans in malignancy and their pharmacological targeting. FEBS J 2010;277(19):3904–3923
3 Theocharis AD, Skandalis SS, Gialeli C, Karamanos NK. Extracellular matrix structure. Adv Drug Deliv Rev 2016;97:4–27
4 Weinbaum S, Tarbell JM, Damiano ER. The structure and function of the endothelial glycocalyx layer. Annu Rev Biomed Eng 2007; 9:121–167
5 Zhang X, Sun D, Song JW, et al. Endothelial cell dysfunction and glycocalyx - a vicious circle. Matrix Biol 2018;71–72:421–431
6 Bennett HS, Luft JH, Hampton JC. Morphological classifications of vertebrate blood capillaries. Am J Physiol 1959;196(02): 381–390
7 Reitsma S, Slaaf DW, Vink H, van Zandvoort MA, oude Egbrink MG. The endothelial glycocalyx: composition, functions, and visualization. Pflugers Arch 2007;454(03):345–359
8 Lennon FE, Singleton PA. Hyaluronan regulation of vascular integrity. Am J Cardiovasc Dis 2011;1(03):200–213
9 Machin DR, Phuong TT, Donato AJ. The role of the endothelial glycocalyx in advanced age and cardiovascular disease. Curr Opin Pharmacol 2019;45:66–71
10 Dogné S, Flamion B, Caron N. Endothelial glycocalyx as a shield against diabetic vascular complications: involvement of hyaluronan and hyaluronidases. Arterioscler Thromb Vasc Biol 2018;38 (07):1427–1439
11 Jourde-Chiche N, Fakhouri F, Dou L, et al. Endothelium structure and function in kidney health and disease. Nat Rev Nephrol 2019;15(02):87–108
12 Iba T, Levy JH. Derangement of the endothelial glycocalyx in sepsis. J Thromb Haemost 2019;17(02):283–294
13 Uchimido R, Schmidt EP, Shapiro NI. The glycocalyx: a novel diagnostic and therapeutic target in sepsis. Crit Care 2019;23(01):16
14 Tuma M, Canestrini S, Alwahab Z, Marshall J. Trauma and endothelial glycocalyx: the microcirculation helmet? Shock 2016;46(04):352–357
15 Becker BF, Jacob M, Leipert S, Salmon AH, Chappell D. Degradation of the endothelial glycocalyx in clinical settings: searching for the sheddases. Br J Clin Pharmacol 2015;80(03):389–402
16 Celie JW, Beelen RH, van den Born J. Heparan sulfate proteoglycans in extravasation: assisting leukocyte guidance. Front Biosci 2009;14:4932–4949
17 Kumar AV, Katakam SK, Urbanowitz AK, Gotte M. Heparan sulphate as a regulator of leukocyte recruitment in inflammation. Curr Protein Pept Sci 2015;16(01):77–86
18 Yang Y, Haeger SM, Suflita MA, et al. Fibroblast growth factor signaling mediates pulmonary endothelial glycocalyx reconstitution. Am J Respir Cell Mol Biol 2017;56(06):727–737
19 Schmidt EP, Li G, Li L, et al. The circulating glycosaminoglycan signature of respiratory failure in critically ill adults. J Biol Chem 2014;289(12):8194–8202
20 Meirovitz A, Goldberg R, Binder A, Rubinstein AM, Hermano E, Elkin M. Heparanase in inflammation and inflammation-associated cancer. FEBS J 2013;280(10):2307–2319
21 Oduah EI, Linhardt RJ, Sharfstein ST. Heparin: past, present, and future. Pharmaceuticals (Basel) 2016;9(03):E38
22 Weiss R, Niecestro R, Raz I. The role of sulodexide in the treatment of diabetic nephropathy. Drugs 2007;67(18): 2681–2696

23 Passi A, Vigetti D, Buraschi S, Iozzo RV. Dissecting the role of hyaluronan synthases in the tumor microenvironment. FEBS J 2019;286(15):2937–2949
24 Vigetti D, Karousou E, Viola M, Deleonibus S, De Luca G, Passi A. Hyaluronan: biosynthesis and signaling. Biochim Biophys Acta 2014;1840(08):2452–2459
25 Girish KS, Kemparaju K. The magic glue hyaluronan and its eraser hyaluronidase: a biological overview. Life Sci 2007;80(21): 1921–1943
26 Bourguignon V, Flamion B. Respective roles of hyaluronidases 1 and 2 in endogenous hyaluronan turnover. FASEB J 2016;30(06): 2108–2114
27 Moseley R, Waddington RJ, Embery G. Degradation of glycosaminoglycans by reactive oxygen species derived from stimulated polymorphonuclear leukocytes. Biochim Biophys Acta 1997; 1362(2–3):221–231
28 Soltés L, Mendichi R, Kogan G, Schiller J, Stankovska M, Arnhold J. Degradative action of reactive oxygen species on hyaluronan. Biomacromolecules 2006;7(03):659–668
29 Rajagopalan S, Meng XP, Ramasamy S, Harrison DG, Galis ZS. Reactive oxygen species produced by macrophage-derived foam cells regulate the activity of vascular matrix metalloproteinases in vitro. Implications for atherosclerotic plaque stability. J Clin Invest 1996;98(11):2572–2579
30 Iijima R, Takahashi H, Namme R, Ikegami S, Yamazaki M. Novel biological function of sialic acid (N-acetylneuraminic acid) as a hydrogen peroxide scavenger. FEBS Lett 2004;561(1–3):163–166
31 Betteridge KB, Arkill KP, Neal CR, et al. Sialic acids regulate microvessel permeability, revealed by novel in vivo studies of endothelial glycocalyx structure and function. J Physiol 2017; 595(15):5015–5035
32 Majerczak J, Duda K, Chlopicki S, et al. Endothelial glycocalyx integrity is preserved in young, healthy men during a single bout of strenuous physical exercise. Physiol Res 2016;65(02): 281–291
33 Majerczak J, Grandys M, Duda K, et al. Moderate-intensity endurance training improves endothelial glycocalyx layer integrity in healthy young men. Exp Physiol 2017;102(01):70–85
34 Afratis NA, Nikitovic D, Multhaupt HA, Theocharis AD, Couchman JR, Karamanos NK. Syndecans - key regulators of cell signaling and biological functions. FEBS J 2017;284(01):27–41
35 Chung H, Multhaupt HA, Oh ES, Couchman JR. Minireview: syndecans and their crucial roles during tissue regeneration. FEBS Lett 2016;590(15):2408–2417
36 Peysselon F, Xue B, Uversky VN, Ricard-Blum S. Intrinsic disorder of the extracellular matrix. Mol Biosyst 2011;7(12):3353–3365
37 Xing Y, Xu Q, Lee C. Widespread production of novel soluble protein isoforms by alternative splicing removal of transmembrane anchoring domains. FEBS Lett 2003;555(03):572–578
38 Couchman JR, Gopal S, Lim HC, Nørgaard S, Multhaupt HA. Fell-Muir lecture: syndecans: from peripheral coreceptors to mainstream regulators of cell behaviour. Int J Exp Pathol 2015;96(01): 1–10
39 Murakami M, Horowitz A, Tang S, Ware JA, Simons M. Protein kinase C (PKC) delta regulates PKCalpha activity in a Syndecan-4-dependent manner. J Biol Chem 2002;277(23):20367–20371
40 Horowitz A, Murakami M, Gao Y, Simons M. Phosphatidylinositol-4,5-bisphosphate mediates the interaction of syndecan-4 with protein kinase C. Biochemistry 1999;38(48):15871–15877
41 Horowitz A, Tkachenko E, Simons M. Fibroblast growth factor-specific modulation of cellular response by syndecan-4. J Cell Biol 2002;157(04):715–725
42 Cheng B, Montmasson M, Terradot L, Rousselle P. Syndecans as cell surface receptors in cancer biology. A focus on their Interaction with PDZ domain proteins. Front Pharmacol 2016;7:10
43 Oh ES, Woods A, Couchman JR. Multimerization of the cytoplasmic domain of syndecan-4 is required for its ability to activate protein kinase C. J Biol Chem 1997;272(18): 11805–11811
44 Chu CL, Buczek-Thomas JA, Nugent MA. Heparan sulphate proteoglycans modulate fibroblast growth factor-2 binding through a lipid raft-mediated mechanism. Biochem J 2004;379(Pt 2):331–341
45 Schmidt A, Echtermeyer F, Alozie A, Brands K, Buddecke E. Plasmin- and thrombin-accelerated shedding of syndecan-4 ectodomain generates cleavage sites at Lys(114)-Arg(115) and Lys(129)-Val(130) bonds. J Biol Chem 2005;280(41): 34441–34446
46 Piperigkou Z, Mohr B, Karamanos N, Götte M. Shed proteoglycans in tumor stroma. Cell Tissue Res 2016;365(03):643–655
47 Morimoto-Tomita M, Uchimura K, Werb Z, Hemmerich S, Rosen SD. Cloning and characterization of two extracellular heparin-degrading endosulfatases in mice and humans. J Biol Chem 2002; 277(51):49175–49185
48 Bishop JR, Schuksz M, Esko JD. Heparan sulphate proteoglycans fine-tune mammalian physiology. Nature 2007;446 (7139):1030–1037
49 Nadanaka S, Kitagawa H. Heparan sulphate biosynthesis and disease. J Biochem 2008;144(01):7–14
50 Qiao D, Meyer K, Mundhenke C, Drew SA, Friedl A. Heparan sulfate proteoglycans as regulators of fibroblast growth factor-2 signaling in brain endothelial cells. Specific role for glypican-1 in glioma angiogenesis. J Biol Chem 2003;278(18):16045–16053
51 Tkachenko E, Rhodes JM, Simons M. Syndecans: new kids on the signaling block. Circ Res 2005;96(05):488–500
52 Kato M, Wang H, Kainulainen V, et al. Physiological degradation converts the soluble syndecan-1 ectodomain from an inhibitor to a potent activator of FGF-2. Nat Med 1998;4(06):691–697
53 Barash U, Cohen-Kaplan V, Dowek I, Sanderson RD, Ilan N, Vlodavsky I. Proteoglycans in health and disease: new concepts for heparanase function in tumor progression and metastasis. FEBS J 2010;277(19):3890–3903
54 Okamoto O, Bachy S, Odenthal U, et al. Normal human keratinocytes bind to the alpha3LG4/5 domain of unprocessed laminin-5 through the receptor syndecan-1. J Biol Chem 2003;278 (45):44168–44177
55 Gondelaud F, Ricard-Blum S. Structures and interactions of syndecans. FEBS J 2019;286(15):2994–3007
56 Filmus J, Selleck SB. Glypicans: proteoglycans with a surprise. J Clin Invest 2001;108(04):497–501
57 Filmus J, Capurro M, Rast J. Glypicans. Genome Biol 2008;9(05): 224
58 Belting M. Heparan sulfate proteoglycan as a plasma membrane carrier. Trends Biochem Sci 2003;28(03):145–151
59 De Cat B, Muyldermans SY, Coomans C, et al. Processing by proprotein convertases is required for glypican-3 modulation of cell survival, Wnt signaling, and gastrulation movements. J Cell Biol 2003;163(03):625–635
60 Traister A, Shi W, Filmus J. Mammalian Notum induces the release of glypicans and other GPI-anchored proteins from the cell surface. Biochem J 2008;410(03):503–511
61 Hippo Y, Watanabe K, Watanabe A, et al. Identification of soluble NH2-terminal fragment of glypican-3 as a serological marker for early-stage hepatocellular carcinoma. Cancer Res 2004;64(07): 2418–2423
62 Filmus J. Glypicans in growth control and cancer. Glycobiology 2001;11(03):19R–23R
63 Rosenberg RD, Shworak NW, Liu J, Schwartz JJ, Zhang L. Heparan sulfate proteoglycans of the cardiovascular system. Specific structures emerge but how is synthesis regulated? J Clin Invest 1997;100(11, Suppl):S67–S75
64 Benjamin EJ, Blaha MJ, Chiuve SE, et al; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics-2017 update: a report from

the American Heart Association. Circulation 2017;135(10): e146–e603
65 Libby P, Buring JE, Badimon L, et al. Atherosclerosis. Nat Rev Dis Primers 2019;5(01):56
66 Lepedda AJ, Formato M. Oxidative modifications in advanced atherosclerotic plaques: a focus on *in situ* protein sulfhydryl group oxidation. Oxid Med Cell Longev 2020;2020:6169825
67 Ostrowski SR, Pedersen SH, Jensen JS, Mogelvang R, Johansson PI. Acute myocardial infarction is associated with endothelial glycocalyx and cell damage and a parallel increase in circulating catecholamines. Crit Care 2013;17(01):R32
68 Solbu MD, Kolset SO, Jenssen TG, et al. Gender differences in the association of syndecan-4 with myocardial infarction: the population-based Tromsø Study. Atherosclerosis 2018;278:166–173
69 Wernly B, Fuernau G, Masyuk M, et al. Syndecan-1 predicts outcome in patients with ST-segment elevation infarction independent from infarct-related myocardial injury. Sci Rep 2019;9(01):18367
70 Jung C, Fuernau G, Muench P, et al. Impairment of the endothelial glycocalyx in cardiogenic shock and its prognostic relevance. Shock 2015;43(05):450–455
71 Tromp J, van der Pol A, Klip IT, et al. Fibrosis marker syndecan-1 and outcome in patients with heart failure with reduced and preserved ejection fraction. Circ Heart Fail 2014;7(03):457–462
72 Bielecka-Dabrowa A, Gluba-Brzózka A, Michalska-Kasiczak M, Misztal M, Rysz J, Banach M. The multi-biomarker approach for heart failure in patients with hypertension. Int J Mol Sci 2015;16 (05):10715–10733
73 Demissei BG, Valente MA, Cleland JG, et al. Optimizing clinical use of biomarkers in high-risk acute heart failure patients. Eur J Heart Fail 2016;18(03):269–280
74 Neves FM, Meneses GC, Sousa NE, et al. Syndecan-1 in acute decompensated heart failure–association with renal function and mortality. Circ J 2015;79(07):1511–1519
75 DellaValle B, Hasseldam H, Johansen FF, Iversen HK, Rungby J, Hempel C. Multiple soluble components of the glycocalyx are increased in patient plasma after ischemic stroke. Stroke 2019; 50(10):2948–2951
76 Miranda CH, de Carvalho Borges M, Schmidt A, Marin-Neto JA, Pazin-Filho A. Evaluation of the endothelial glycocalyx damage in patients with acute coronary syndrome. Atherosclerosis 2016; 247:184–188
77 Zoungas S, Woodward M, Li Q, et al; ADVANCE Collaborative group. Impact of age, age at diagnosis and duration of diabetes on the risk of macrovascular and microvascular complications and death in type 2 diabetes. Diabetologia 2014;57(12):2465–2474
78 Chatterjee S, Khunti K, Davies MJ. Type 2 diabetes. Lancet 2017; 389(10085):2239–2251
79 Nieuwdorp M, van Haeften TW, Gouverneur MC, et al. Loss of endothelial glycocalyx during acute hyperglycemia coincides with endothelial dysfunction and coagulation activation in vivo. Diabetes 2006;55(02):480–486
80 Nieuwdorp M, Mooij HL, Kroon J, et al. Endothelial glycocalyx damage coincides with microalbuminuria in type 1 diabetes. Diabetes 2006;55(04):1127–1132
81 Svennevig K, Kolset SO, Bangstad HJ. Increased syndecan-1 in serum is related to early nephropathy in type 1 diabetes mellitus patients. Diabetologia 2006;49(09):2214–2216
82 Kolseth IB, Reine TM, Parker K, et al. Increased levels of inflammatory mediators and proinflammatory monocytes in patients with type I diabetes mellitus and nephropathy. J Diabetes Complications 2017;31(01):245–252
83 Wang JB, Zhang YJ, Zhang Y, et al. Negative correlation between serum syndecan-1 and apolipoprotein A1 in patients with type 2 diabetes mellitus. Acta Diabetol 2013;50(02):111–115
84 Padberg JS, Wiesinger A, di Marco GS, et al. Damage of the endothelial glycocalyx in chronic kidney disease. Atherosclerosis 2014;234(02):335–343
85 Vlahu CA, Lemkes BA, Struijk DG, Koopman MG, Krediet RT, Vink H. Damage of the endothelial glycocalyx in dialysis patients. J Am Soc Nephrol 2012;23(11):1900–1908
86 Koch J, Idzerda NMA, Dam W, Assa S, Franssen CFM, van den Born J. Plasma syndecan-1 in hemodialysis patients associates with survival and lower markers of volume status. Am J Physiol Renal Physiol 2019;316(01):F121–F127
87 Dane MJ, Khairoun M, Lee DH, et al. Association of kidney function with changes in the endothelial surface layer. Clin J Am Soc Nephrol 2014;9(04):698–704
88 Adepu S, Rosman CW, Dam W, et al. Incipient renal transplant dysfunction associates with tubular syndecan-1 expression and shedding. Am J Physiol Renal Physiol 2015;309(02):F137–F145
89 Lepedda AJ, De Muro P, Capobianco G, Formato M. Significance of urinary glycosaminoglycans/proteoglycans in the evaluation of type 1 and type 2 diabetes complications. J Diabetes Complications 2017;31(01):149–155
90 Nakamura K, Fuster JJ, Walsh K. Adipokines: a link between obesity and cardiovascular disease. J Cardiol 2014;63(04):250–259
91 Gesta S, Blüher M, Yamamoto Y, et al. Evidence for a role of developmental genes in the origin of obesity and body fat distribution. Proc Natl Acad Sci U S A 2006;103(17):6676–6681
92 Ussar S, Bezy O, Blüher M, Kahn CR. Glypican-4 enhances insulin signaling via interaction with the insulin receptor and serves as a novel adipokine. Diabetes 2012;61(09):2289–2298
93 Flehmig G, Scholz M, Klöting N, et al. Identification of adipokine clusters related to parameters of fat mass, insulin sensitivity and inflammation. PLoS One 2014;9(06):e99785
94 Zhu HJ, Pan H, Cui Y, et al. The changes of serum glypican4 in obese patients with different glucose metabolism status. J Clin Endocrinol Metab 2014;99(12):E2697–E2701
95 Leelalertlauw C, Korwutthikulrangsri M, Mahachoklertwattana P, et al. Serum glypican 4 level in obese children and its relation to degree of obesity. Clin Endocrinol (Oxf) 2017;87(06):689–695
96 El-Serag HB. Hepatocellular carcinoma. N Engl J Med 2011;365 (12):1118–1127
97 Hsu HC, Cheng W, Lai PL. Cloning and expression of a developmentally regulated transcript MXR7 in hepatocellular carcinoma: biological significance and temporospatial distribution. Cancer Res 1997;57(22):5179–5184
98 Li J, Gao JZ, Du JL, Wei LX. Prognostic and clinicopathological significance of glypican-3 overexpression in hepatocellular carcinoma: a meta-analysis. World J Gastroenterol 2014;20(20): 6336–6344
99 Xiao WK, Qi CY, Chen D, et al. Prognostic significance of glypican-3 in hepatocellular carcinoma: a meta-analysis. BMC Cancer 2014;14:104
100 Liu H, Yang C, Lu W, Zeng Y. Prognostic significance of glypican-3 expression in hepatocellular carcinoma: a meta-analysis. Medicine (Baltimore) 2018;97(04):e9702
101 Zhang J, Zhang M, Ma H, et al. Overexpression of glypican-3 is a predictor of poor prognosis in hepatocellular carcinoma: an updated meta-analysis. Medicine (Baltimore) 2018;97(24): e11130
102 Moudi B, Heidari Z, Mahmoudzadeh-Sagheb H. Meta-analysis and systematic review of prognostic significance of Glypican-3 in patients with hepatitis B-related hepatocellular carcinoma. Virusdisease 2019;30(02):193–200
103 Zhou F, Shang W, Yu X, Tian J. Glypican-3: a promising biomarker for hepatocellular carcinoma diagnosis and treatment. Med Res Rev 2018;38(02):741–767
104 Guo M, Zhang H, Zheng J, Liu Y. Glypican-3: a new target for diagnosis and treatment of hepatocellular carcinoma. J Cancer 2020;11(08):2008–2021
105 Liu XF, Hu ZD, Liu XC, Cao Y, Ding CM, Hu CJ. Diagnostic accuracy of serum glypican-3 for hepatocellular carcinoma: a systematic review and meta-analysis. Clin Biochem 2014;47(03):196–200

106 Yang SL, Fang X, Huang ZZ, et al. Can serum glypican-3 be a biomarker for effective diagnosis of hepatocellular carcinoma? A meta-analysis of the literature. Dis Markers 2014;2014:127831
107 Jia X, Liu J, Gao Y, Huang Y, Du Z. Diagnosis accuracy of serum glypican-3 in patients with hepatocellular carcinoma: a systematic review with meta-analysis. Arch Med Res 2014;45(07):580–588
108 Liu JW, Zuo XL, Wang S. Diagnosis accuracy of serum glypican-3 level in patients with hepatocellular carcinoma and liver cirrhosis: a meta-analysis. Eur Rev Med Pharmacol Sci 2015;19(19): 3655–3673
109 Xu D, Su C, Sun L, Gao Y, Li Y. Performance of serum glypican 3 in diagnosis of hepatocellular carcinoma: a meta-analysis. Ann Hepatol 2019;18(01):58–67
110 Attallah AM, El-Far M, Omran MM, et al. GPC-HCC model: a combination of glybican-3 with other routine parameters improves the diagnostic efficacy in hepatocellular carcinoma. Tumour Biol 2016;37(09):12571–12577
111 Ofuji K, Saito K, Suzuki S, et al. Perioperative plasma glypican-3 level may enable prediction of the risk of recurrence after surgery in patients with stage I hepatocellular carcinoma. Oncotarget 2017;8(23):37835–37844
112 Shimizu Y, Mizuno S, Fujinami N, et al. Plasma and tumoral glypican-3 levels are correlated in patients with hepatitis C virus-related hepatocellular carcinoma. Cancer Sci 2020;111 (02):334–342
113 Hideshima T, Mitsiades C, Tonon G, Richardson PG, Anderson KC. Understanding multiple myeloma pathogenesis in the bone marrow to identify new therapeutic targets. Nat Rev Cancer 2007;7(08):585–598
114 Akl MR, Nagpal P, Ayoub NM, et al. Molecular and clinical profiles of syndecan-1 in solid and hematological cancer for prognosis and precision medicine. Oncotarget 2015;6(30):28693–28715
115 Ramani VC, Purushothaman A, Stewart MD, et al. The heparanase/syndecan-1 axis in cancer: mechanisms and therapies. FEBS J 2013;280(10):2294–2306
116 Tripathi[Q9] K, Ramani VC, Bandari SK, et al. Heparanase promotes myeloma stemness and in vivo tumorigenesis. Matrix Biol 2020; 88:53–68
117 Reijmers RM, Spaargaren M, Pals ST. Heparan sulfate proteoglycans in the control of B cell development and the pathogenesis of multiple myeloma. FEBS J 2013;280(10):2180–2193
118 Mahtouk K, Cremer FW, Rème T, et al. Heparan sulphate proteoglycans are essential for the myeloma cell growth activity of EGF-family ligands in multiple myeloma. Oncogene 2006;25 (54):7180–7191
119 Mahtouk K, Hose D, Raynaud P, et al. Heparanase influences expression and shedding of syndecan-1, and its expression by the bone marrow environment is a bad prognostic factor in multiple myeloma. Blood 2007;109(11):4914–4923
120 Purushothaman A, Uyama T, Kobayashi F, et al. Heparanase-enhanced shedding of syndecan-1 by myeloma cells promotes endothelial invasion and angiogenesis. Blood 2010;115(12):2449–2457
121 Purushothaman A, Sanderson RD. Heparanase: a dynamic promoter of myeloma progression. Adv Exp Med Biol 2020;1221:331–349
122 Ritchie JP, Ramani VC, Ren Y, et al. SST0001, a chemically modified heparin, inhibits myeloma growth and angiogenesis via disruption of the heparanase/syndecan-1 axis. Clin Cancer Res 2011;17(06):1382–1393
123 Seidel C, Sundan A, Hjorth M, et al. Serum syndecan-1: a new independent prognostic marker in multiple myeloma. Blood 2000;95(02):388–392
124 Bjøro B, Dalgard O, Midgard H, Verbaan H, Småstuen MC, Rustøen T. Increased hope following successful treatment for hepatitis C infection. J Adv Nurs 2018;74(03):724–733
125 Aref S, Goda T, El-Sherbiny M. Syndecan-1 in multiple myeloma: relationship to conventional prognostic factors. Hematology 2003;8(04):221–228
126 Jánosi J, Sebestyén A, Mikala G, Németh J, Kiss Z, Vályi-Nagy I. Soluble syndecan-1 levels in different plasma cell dyscrasias and in different stages of multiple myeloma. Haematologica 2004;89 (03):370–371
127 Lovell R, Dunn JA, Begum G, et al; Working Party on Leukaemia in Adults of the National Cancer Research Institute Haematological Oncology Clinical Studies Group. Soluble syndecan-1 level at diagnosis is an independent prognostic factor in multiple myeloma and the extent of fall from diagnosis to plateau predicts for overall survival. Br J Haematol 2005;130(04):542–548
128 Yang Y, MacLeod V, Dai Y, et al. The syndecan-1 heparan sulfate proteoglycan is a viable target for myeloma therapy. Blood 2007; 110(06):2041–2048
129 Katz BZ. Adhesion molecules–the lifelines of multiple myeloma cells. Semin Cancer Biol 2010;20(03):186–195
130 Vassilakopoulos TP, Kyrtsonis MC, Papadogiannis A, et al. Serum levels of soluble syndecan-1 in Hodgkin's lymphoma. Anticancer Res 2005;25(6C):4743–4746
131 Grindel B, Li Q, Arnold R, et al. Perlecan/HSPG2 and matrilysin/MMP-7 as indices of tissue invasion: tissue localization and circulating perlecan fragments in a cohort of 288 radical prostatectomy patients. Oncotarget 2016;7(09):10433–10447
132 Levin RA, Lund ME, Truong Q, et al. Development of a reliable assay to measure glypican-1 in plasma and serum reveals circulating glypican-1 as a novel prostate cancer biomarker. Oncotarget 2018;9(32):22359–22367
133 Szarvas T, Sevcenco S, Modos O, et al. Circulating syndecan-1 is associated with chemotherapy-resistance in castration-resistant prostate cancer. Urol Oncol 2018;36(06):312.e9–312.e15
134 Lai X, Wang M, McElyea SD, Sherman S, House M, Korc M. A microRNA signature in circulating exosomes is superior to exosomal glypican-1 levels for diagnosing pancreatic cancer. Cancer Lett 2017;393:86–93
135 Melo SA, Luecke LB, Kahlert C, et al. Glypican-1 identifies cancer exosomes and detects early pancreatic cancer. Nature 2015;523 (7559):177–182
136 Joensuu H, Anttonen A, Eriksson M, et al. Soluble syndecan-1 and serum basic fibroblast growth factor are new prognostic factors in lung cancer. Cancer Res 2002;62(18):5210–5217
137 Wołowiec D, Dybko J, Wróbel T, et al. Circulating sCD138 and some angiogenesis-involved cytokines help to anticipate the disease progression of early-stage B-cell chronic lymphocytic leukemia. Mediators Inflamm 2006;2006(03):42394
138 Jilani I, Wei C, Bekele BN, et al. Soluble syndecan-1 (sCD138) as a prognostic factor independent of mutation status in patients with chronic lymphocytic leukemia. Int J Lab Hematol 2009;31 (01):97–105
139 Li J, Chen Y, Guo X, et al. GPC1 exosome and its regulatory miRNAs are specific markers for the detection and target therapy of colorectal cancer. J Cell Mol Med 2017;21(05):838–847
140 Zhou S, O'Gorman MR, Yang F, Andresen K, Wang L. Glypican 3 as a serum marker for hepatoblastoma. Sci Rep 2017;7:45932
141 Esko JD, Selleck SB. Order out of chaos: assembly of ligand binding sites in heparan sulfate. Annu Rev Biochem 2002; 71:435–471

Supplementary Table S1 Main findings of studies on the association of serum HS-PGs levels with cardiovascular disease, performed in the last 20 years

Serum proteoglycans/ GAGs	CVD typology	Casuistry	Main findings	Reference
Syndecan-1, -2, -3, -4	Postmyocardial infarction	23 mice: syndecan-1 wild-type (WT) Sham ($n=4$); syndecan-1 KO Sham ($n=5$); syndecan-1 WT MI ($n=7$); syndecan-1 KO MI ($n=7$)	Transcript levels of syndecans: syndecan-1 levels: Synd1 WT Sham (1.6 ± 0.13); Synd1 KO Sham (ND); Synd1 WT MI (14 ± 1.3); Synd1 KO MI (ND). Syndecan-2 levels: Synd1 WT Sham (0.4 ± 0.02); Synd1 KO Sham (0.35 ± 0.06); Synd1 WT MI (0.96 ± 0.08); Synd1 KO MI (0.92 ± 0.10). Syndecan-3 levels: Synd1 WT Sham (0.48 ± 0.03); Synd1 KO Sham (0.39 ± 0.03); Synd1 WT MI (1.1 ± 0.17); Synd1 KO MI (0.73 ± 0.07). Syndecan-4 levels: Synd1 WT Sham (0.43 ± 0.05); Synd1 KO Sham (0.39 ± 0.06); Synd1 WT MI (1.2 ± 0.12); Synd1 KO MI (1.0 ± 0.16)	Vanhoutte D, Schellings MW, Götte M, et al. Increased expression of syndecan-1 protects against cardiac dilatation and dysfunction after myocardial infarction. Circulation. 2007;115(4):475–482. doi:10.1161/CIRCULATIONAHA.106.644609
Syndecan-4	Chronic heart failure	41 patients vs. 21 healthy subjects	Patients: 22.5 ± 12.3 ng/mL vs. controls: 5.7 ± 3.3 ng/mL	Takahashi R, Negishi K, Watanabe A, et al. Serum syndecan-4 is a novel biomarker for patients with chronic heart failure. J Cardiol. 2011;57(3):325–332. doi:10.1016/j.jjcc.2011.01.012
Syndecan-4 (syndecan-1, -2, -3)	Postmyocardial infarction	66 WT mice vs. 56 Syn4 −/− mice	Syndecan-4 protein levels were elevated immediately after MI and decreased gradually. There was no significant difference in mRNA expression of syndecan-1, syndecan-2, and syndecan-3 between WT and syndecan-4 −/− mice at baseline. Transcript levels of syndecan-1, syndecan-2, and syndecan-3 were increased significantly in WT and syndecan-4 −/− infarcts 4 days after MI compared with sham-operated hearts. Syndecan-4 deficiency led to the decreased expression of syndecan-3, but not syndecan-1 or syndecan-2, compared with WT mice at day 4 after MI.	Matsui Y, Ikesue M, Danzaki K, et al. Syndecan-4 prevents cardiac rupture and dysfunction after myocardial infarction. Circ Res. 2011;108(11):1328–1339. doi:10.1161/CIRCRESAHA.110.235689
Syndecan-1, HS	Ischemia/ reperfusion injury	10 patients	No time-dependent change in levels of HS at baseline (6.5 ± 1.9 μg/mL), 0 minute reperfusion (5.6 ± 1.9 μg/mL), 2 minute reperfusion (5.5 ± 2.1 μg/mL), and 10 minute reperfusion (6.2 ± 1.8 μg/mL). Syndecan-1 levels showed no time dependent changes from baseline (196.4 ± 28.4 ng/mL) to 0 minute reperfusion (196.5 ± 35.8 ng/mL), 2 minute reperfusion (196.0 ± 18.6 ng/mL), and 10 minute reperfusion (102.7 ± 27.5 ng/mL).	Kamat P, Juon B, Jossen B, Gajanayake T, Rieben R, Vögelin E. Assessment of endothelium and inflammatory response at the onset of reperfusion injury in hand surgery. J Inflamm (Lond). 2012;9(1):18. Published 2012 May 14. doi:10.1186/1476-9255-9-18
Syndecan-1, HS, HA	Postcardiac arrest syndrome	25 patients after immediate survival of cardiac arrest vs. 12 patients (controls) with acute coronary syndrome	Increase of syndecan-1 in the early phase of PCAS (2.8-fold increase vs. controls) and a later peak of heparan sulfate (1.7-fold increase) and hyaluronic acid (2-fold increase) in the intermediate phase	Grundmann S, Fink K, Rabadzhieva L, et al. Perturbation of the endothelial glycocalyx in post cardiac arrest syndrome. Resuscitation. 2012;83(6):715–720. doi:10.1016/j.resuscitation.2012.01.028
Syndecan-1	Acute myocardial infarction	571 ST-segment elevation myocardial infarction (STEMI) patients divided into Adrenaline Q1 ($n=143$), Adrenaline Q2 ($n=143$), Adrenaline Q3 ($n=142$), and Adrenaline Q4 ($n=143$).	Syndecan-1 baseline levels: all patients: 92 (52–165); Q1: 84 (46–156); Q2: 81 (47–162); Q3: 95 (55–168); Q4: 101 (52–169) ng/mL. With increasing syndecan-1 quartile (33, 69, 123, and 248 ng/mL in Q1 to Q4, respectively), more patients had shock prior to PCI.	Ostrowski SR, Pedersen SH, Jensen JS, Mogelvang R, Johansson PI. Acute myocardial infarction is associated with endothelial glycocalyx and cell damage and a parallel increase in circulating catecholamines. Crit Care. 2013;17(1):R32. Published 2013 Feb 22. doi:10.1186/cc12532

Supplementary Table S1 (*Continued*)

Serum proteoglycans/ GAGs	CVD typology	Casuistry	Main findings	Reference
Syndecan-4	Dilated cardiomyopathy	45 patients with left ventricular ejection fraction $\leq$40% treated with optimal medical therapy	Baseline syndecan-4 (ng/mL) 4.7 ± 3.52. After 5-year follow-up it correlated negatively with left ventricular ejection fraction and positively with the lower left ventricular systolic diameter and higher left ventricular diastolic diameter. There was no dependence of syndecan-4 concentration on BMI, gender, or age.	Bielecka-Dabrowa A, von Haehling S, Aronow WS, Ahmed MI, Rysz J, Banach M. Heart failure biomarkers in patients with dilated cardiomyopathy. Int J Cardiol. 2013;168 (3):2404–2410. doi:10.1016/j.ijcard.2013.01.157
Syndecan-1	Heart failure	567 patients with chronic heart failure	Baseline syndecan-1: 2.4–393.0ng/mL. Patients' median syndecan-1 levels were 20.1 ng/mL.	Tromp J, van der Pol A, Klip IT, et al. Fibrosis marker syndecan-1 and outcome in patients with heart failure with reduced and preserved ejection fraction. Circ Heart Fail. 2014;7(3):457–462. doi:10.1161/CIRCHEARTFAILURE.113.000846
Syndecan-1, HS	Cardiogenic shock	184 patients with cardiogenic shock complicating acute myocardial infarction	Levels of syndecan-1 decreased between days 1 and 2 (339 vs. 220 ng/mL). Syndecan-1 in healthy subjects was 20 ng/mL, significantly lower compared with patients with cardiogenic shock. In contrast, glycosaminoglycan heparan sulfate increased over time (1.9 vs. 7.1 mg/mL).	Jung C, Fuernau G, Muench P, et al. Impairment of the endothelial glycocalyx in cardiogenic shock and its prognostic relevance [published correction appears in Shock. 2016 Apr;45(4):469] [published correction appears in Shock. 2016 Jan 29]. *Shock*. 2015;43(5):450–455. doi:10.1097/SHK.0000000000000329
Syndecan-1	Cardiac arrest	163 patients resuscitated after out-of-hospital cardiac arrest	ST segment elevation myocardial infarction-induced out-of-hospital cardiac arrest patients had almost 3-fold higher syndecan-1 levels compared with patients with other causes of out-of-hospital cardiac arrest (median 206 vs. 81 ng/mL). Admission mean levels of syndecan-1: 152 ng/mL.	Johansson PI, Bro-Jeppesen J, Kjaergaard J, Wanscher M, Hassager C, Ostrowski SR. Sympathoadrenal activation and endothelial damage are inter correlated and predict increased mortality in patients resuscitated after out-of-hospital cardiac arrest. a post Hoc sub-study of patients from the TTM-trial. PLoS One. 2015;10(3):e0120914. Published 2015 Mar 19. doi:10.1371/journal.pone.0120914
Syndecan-1	Acute decompensated heart failure	201 patients with acute decompensated heart failure divided into: normal renal function ($n = 96$), stable chronic kidney disease ($n = 43$), and acute kidney injury ($n = 62$)	Syndecan-1 levels: normal renal function 91.4 ± 42.9 ng/mL, stable chronic kidney disease 98.1 ± 65.6ng/mL, acute kidney injury 248.7 ± 165.6 ng/mL. Acute Decompensated Heart Failure mean level of serum syndecan-1 $= 133.7 \pm 95.0$ ng/mL.	Neves FM, Meneses GC, Sousa NE, et al. Syndecan-1 in Acute Decompensated Heart Failure–Association With Renal Function and Mortality. *Circ J*. 2015;79(7):1511–1519. doi:10.1253/circj.CJ-14-1195
Syndecan-4	Heart failure	120 hypertensive patients with ($n = 60$) vs. without ($n = 60$) overt heart failure	Syndecan (ng/mL) heart failure 1.39 ± 1.08 vs. non-heart failure 4.14 ± 3. Syndecan > 2.3 ng/mL is a significant predictor of overt heart failure in patients with hypertension	Bielecka-Dabrowa A, Gluba-Brzózka A, Michalska-Kasiczak M, Misztal M, Rysz J, Banach M. The multi-biomarker approach for heart failure in patients with hypertension. Int J Mol Sci. 2015;16(5):10715–10733. Published 2015 May 12. doi:10.3390/ijms160510715
Syndecan-1	Acute heart failure	2033 patients with acute heart failure	Syndecan-1 showed a significant interaction with ejection fraction status. Baseline syndecan-1 $= 8.3$ ng/mL (7.0–10.1).	Demissei BG, Valente MA, Cleland JG, et al. Optimizing clinical use of biomarkers in high-risk acute heart failure patients. Eur J Heart Fail. 2016;18(3):269–280. doi:10.1002/ejhf.443
Syndecan-1, HS	Hemorrhaged rats after different resuscitation fluids	8 control rats vs. 46 hemorrhaged animals subjected to 7 resuscitation fluids	Baseline: syndecan-1 ($n = 58$) 59.9 ± 9.0 ng/mL; heparan sulfate proteoglycan ($n = 30$) 288.6 ± 18.3 ng/mL.	Torres Filho IP, Torres LN, Salgado C, Dubick MA. Plasma syndecan-1 and heparan sulfate correlate with microvascular glycocalyx degradation in hemorrhaged rats after different resuscitation fluids. Am J Physiol Heart Circ Physiol. 2016;310(11): H1468–H1478. doi:10.1152/ajpheart.00006.2016

(*Continued*)

Supplementary Table S1 (*Continued*)

Serum proteoglycans/ GAGs	CVD typology	Casuistry	Main findings	Reference
Syndecan-1	Cerebral ischemia following aneurysmal subarachnoid hemorrhage	3 patients during active delayed cerebral ischemia (DCI) following subarachnoid hemorrhage (SAH) vs. 11 healthy control samples	Syndecan-1 levels: aneurysmal subarachnoid hemorrhage vs. controls 1.18 ± 0.52 fold increase. Delayed cerebral ischemia vs. PRE-delayed cerebral ischemia (PLASMA) 1.41 ± 0.14 fold higher.	Bell JD, Rhind SG, Di Battista AP, Macdonald RL, Baker AJ. Biomarkers of glycocalyx injury are associated with delayed cerebral ischemia following aneurysmal subarachnoid hemorrhage: a case series supporting a new hypothesis. Neurocrit Care. 2017;26(3):339–347. doi:10.1007/s12028-016-0357-4
Syndecan-1	Takotsubo cardiomyopathy (TTC)	48 TTC (acutely and after 3 months) vs. 12 healthy controls, and 17 patients with acute myocardial infarction	Acute syndecan-1 concentrations in TTC patients were elevated significantly compared with control (mean 97 ± 65 vs. 41 ± 10 µg/L). Syndecan-1 levels were lower in acute TTC compared with acute MI patients (mean 97 ± 65 vs. 256 ± 208 µg/L).	Nguyen TH, Liu S, Ong GJ, Stafford I, Frenneaux MP, Horowitz JD. Glycocalyx shedding is markedly increased during the acute phase of Takotsubo cardiomyopathy. Int J Cardiol. 2017;243:296–299. doi:10.1016/j.ijcard.2017.04.085
Syndecan-1 and -4	Myocardial infarction	1,495 participants from the Tromsø Study (665 patients vs. 830 controls)	Baseline syndecan-1 levels: patients 42.45 ng/mL (27.97–59.75) vs. controls 42.20 ng/mL (29.23–62.32); baseline syndecan-4 levels: patients 18.45 ng/mL vs. controls 18.73 - ng/mL. Syndecan-4 was associated with incident MI, but not ischemic stroke, and in gender-specific analyses, this association was seen in women only. Syndecan-1 was not associated with any event.	Solbu MD, Kolset SO, Jenssen TG, et al. Gender differences in the association of syndecan-4 with myocardial infarction: the population-based Tromsø Study. Atherosclerosis. 2018;278:166–173. doi:10.1016/j.atherosclerosis.2018.08.005
Syndecan-1	STEMI patients developing cardiogenic shock	STEMI patients with admission cardiogenic shock ($n = 85$) and cardiogenic shock developed in the catheterization laboratory ($n = 25$) vs. noncardiogenic shock patients ($n = 1299$).	Plasma level of syndecan-1 in the overall cohort: admission-cardiogenic shock: 147 ng/mL (109–162); cardiogenic shock developed in the catheterization laboratory: 129 ng/mL (82–160); late cardiogenic shock: 107 ng/mL (67–141); noncardiogenic shock: 92 ng/mL (62–138).	Frydland M, Ostrowski SR, Møller JE, et al. Plasma concentration of biomarkers reflecting endothelial cell- and glycocalyx damage are increased in patients with suspected ST-elevation myocardial infarction complicated by cardiogenic shock. Shock. 2018;50 (5):538–544. doi:10.1097/SHK.0000000000001123
Syndecan-1, HA, HS	Pulmonary artery hypertension	15 animals randomized into 3 groups: monocrotaline-induced pulmonary artery hypertension ($n = 5$), control group ($n = 5$), and heparin-treated group ($n = 5$).	Rats in MCT group had higher plasma HSPG, HA, and syndecan-1 levels than the control group. On the contrary, rats in the treatment group showed reduced plasma levels of HSPG, HA, and syndecan-1 when compared with the MCT group. The protein expression of HSPG and syndecan-1 was significantly reduced in pulmonary arteries in rats from the MCT group than the control group. Interestingly, the levels of HSPG and syndecan-1 protein from rats in the treatment group were significantly higher than those from the MCT group. In our study, the rat model of pulmonary hypertension induced by MCT showed significantly higher levels of peripheral blood glycocalyx shedding ingredients, such as HSPG, HA, and syndecan-1, when compared with control groups.	Guo J, Yang ZC, Liu Y. Attenuating pulmonary hypertension by protecting the integrity of glycocalyx in rats model of pulmonary artery hypertension. inflammation. 2019;42(6):1951–1956. doi:10.1007/s10753-019-01055-5
Syndecan-1, -2, -3, -4, CS, KS, HA, CD44, glypican-1, and biglycan	Ischemic stroke	Plasma samples from healthy individuals ($n = 8$) vs. patients with ischemic stroke ($n = 14$) (within 72 hours [$n = 13$], day 7 [$n = 9$], and day 90 [$n = 13$]).	KS, CS, and HS as well as PG CD44 levels were all above healthy individuals' levels at day 7, and returned to healthy individuals' levels at day 90. Day 7 KS and CD44 levels were above day ≤ 3 and day 90, and CS levels at day 7 were above day 90. Syndecan-3 was significantly increased at day ≤ 3 and day 7. Syndecan-2 was significantly reduced; syndecan-3 was significantly increased at day ≤ 3 and day	DellaValle B, Hasseldam H, Johansen FF, Iversen HK, Rungby J, Hempel C. Multiple soluble components of the glycocalyx are increased in patient plasma after ischemic stroke. Stroke. 2019;50(10):2948–2951. doi:10.1161/STROKEAHA.119.025953

Supplementary Table S1 (*Continued*)

Serum proteoglycans/ GAGs	CVD typology	Casuistry	Main findings	Reference
			7; syndecan-1, syndecan-4, GPC-1, and biglycan did not vary significantly.	
Syndecan-1, HA	Cardiac arrest and resuscitation (postcardiac arrest syndrome)	15 patients after prehospital resuscitation (survivors $n=9$, nonsurvivors $n=6$).	Syndecan-1 serum levels were higher in nonsurvivors 0–12 hours after cardiac arrest. 24 and 48 hours after cardiac arrest, syndecan-1 release was higher in survivors. Syndecan-1 serum levels were higher in patients suffering from multiple organ failure 0–48 hours after cardiac arrest. Significant increase of hyaluronan levels within 48 hours after resuscitation.	Bogner-Flatz V, Braunstein M, Ocker LE, et al. On-the-scene hyaluronan and syndecan-1 serum concentrations and outcome after cardiac arrest and resuscitation. Mediators Inflamm. 2019;2019:8071619. Published 2019 Apr 17. doi:10.1155/2019/8071619
Syndecan-1	STEMI	206 patients admitted for STEMI (followed-up for 6 months) vs. 20 healthy controls.	Patients sdc1 was 62 ng/mL (median) vs. the healthy controls 11 ng/mL.	Wernly B, Fuernau G, Masyuk M, et al. Syndecan-1 predicts outcome in patients with ST-segment elevation infarction independent from infarct-related myocardial injury. Sci Rep. 2019;9(1):18367. Published 2019 Dec 4. doi:10.1038/s41598-019-54937-x
Syndecan-1	TTC and STEMI	20 TTC patients vs. 40 STEMI patients	No differences in syndecan-1 concentrations between the groups were identified: 77 (35–111) vs. 81 (58–121) ng/mL	Højagergaard MA, Hassager C, Christensen TE, et al. Biomarkers in patients with Takotsubo cardiomyopathy compared with patients with acute anterior ST-elevation myocardial infarction. Biomarkers. 2020;25(2):137–143. doi:10.1080/1354750-X.2019.1710767
Syndecan-1	Chronic heart failure with reduced ejection fraction	26 patients with dyssynchronous heart failure with reduced ejection fraction before vs. 6 months after CRT insertion.	Baseline syndecan-1 levels: males had statistically significantly higher syndecan-1 levels compared with females. Levels of syndecan-1 were not elevated beyond the normal range (median 20.1 ng/mL)	Ajaero CN, Procter NEK, Chirkov YY, et al. Endothelial dysfunction and glycocalyx shedding in heart failure: insights from patients receiving cardiac resynchronisation therapy. Heart Vessels. 2020;35(2):197–206. doi:10.1007/s00380-019-01481-3
Syndecan-1	Hemorrhagic shock and resuscitation	32 patients vs. normal donors	Significantly elevated levels of syndecan-1 (554 ± 93 ng/mL) in patients with hemorrhagic shock after injury, which decreased with resuscitation (187 ± 36 ng/mL) but was elevated compared with normal donors (27 ± 1 ng/mL).	Haywood-Watson RJ, Holcomb JB, Gonzalez EA, et al. Modulation of syndecan-1 shedding after hemorrhagic shock and resuscitation. PLoS One. 2011;6(8):e23530. doi:10.1371/journal.pone.0023530
Syndecan-1	Hemorrhagic shock and resuscitation	121 microvessels from cremaster muscle were studied in 32 anesthetized instrumented rats sorted in five experimental groups (~6 rats per group) [(1) HEM, hemorrhage only; (2) LR, resuscitation with LR, dose of 75 mL/kg (3× shed blood); (3) HEX, resuscitation with HEX, dose of 15 mL/kg; (4) FFP, resuscitation with fresh frozen platelet-poor plasma, dose of 15 mL/kg; (5) Sham animals were subjected to all procedures except hemorrhage and resuscitation].	Syndecan-1 was unchanged in SHAM animals throughout the experimental period and similar to baseline values (5.33 ± 1.98 µg/dL). Non-treated rats (HEM group) had significantly higher levels of plasma syndecan, as well as rats treated with LR and HEX (although only the LR group was significantly higher compared with the FFP group). The FFP group restored syndecan to baseline levels and therefore was lower compared with the HEM group.	Torres LN, Sondeen JL, Ji L, Dubick MA, Torres Filho I. Evaluation of resuscitation fluids on endothelial glycocalyx, venular blood flow, and coagulation function after hemorrhagic shock in rats. J Trauma Acute Care Surg. 2013;75(5):759–766. doi:10.1097/TA.0b013e3182a92514
Syndecan-1	Acute coronary syndrome	141 patients (99 men) with ACS compared with those of 45 patients (24 men) with noncoronary chest pain (NCCP) vs. 24 (14 men) healthy individuals (CONTROL).	Syndecan-1 levels: ACS (77 ng/mL), NCCP (60 ng/mL), CONTROL group (42 ng/mL). A syndecan-1 level higher than 148 ng/mL was associated with ACS diagnosis.	Miranda CH, de Carvalho Borges M, Schmidt A, Marin-Neto JA, Pazin-Filho A. Evaluation of the endothelial glycocalyx damage in patients with acute coronary syndrome. Atherosclerosis. 2016;247:184–188. doi:10.1016/j.atherosclerosis.2016.02.023

(*Continued*)

Supplementary Table S1 (*Continued*)

Serum proteoglycans/ GAGs	CVD typology	Casuistry	Main findings	Reference
Syndecan-2 and -4	Hemorrhagic shock	64 WT and syndecan-1 −/− mice (8 groups, 8 mice each). Each group is WT or syndecan 1 −/− (Sham, HS = trauma and hemorrhagic shock; FFP = fresh frozen plasma; and LR = lactated ringers)	Loss of syndecan-1 in vivo leads to equivalency between LR and FFP in restoring pulmonary injury, inflammation, and permeability after shock. Lastly, syndecan-1 −/− mice demonstrated a significant increase in pulmonary syndecan-4 expression after hemorrhagic shock and FFP-based resuscitation.	Wu F, Peng Z, Park PW, Kozar RA. Loss of syndecan-1 abrogates the pulmonary protective phenotype induced by plasma after hemorrhagic shock. Shock. 2017;48 (3):340–345. doi:10.1097/ SHK.0000000000000832
Syndecan-1	Hemorrhagic shock	32 mice (Sham, HS = trauma and hemorrhagic shock; FFP = fresh frozen plasma; and LR = lactated ringers).	Higher postresuscitation syndecan-1 levels correlated with a higher mortality.	Peng Z, Pati S, Potter D, et al. Fresh frozen plasma lessens pulmonary endothelial inflammation and hyperpermeability after hemorrhagic shock and is associated with loss of syndecan 1. Shock. 2013;40 (3):195–202. doi:10.1097/ SHK.0b013e31829f91fc
Syndecan-1, HS	Major vascular (aortic) surgery	32 patients with aortic surgery (12 surgeries of the ascending aorta with CPB and DHCA, 6 only CPB during repair of an aortic aneurysm and/or the aortic valve, and 14 surgeries for an infrarenal aortic aneurysm without CPB) vs. 10 healthy controls	Basal values of syndecan-1 (1.2 μg/dL) and heparan sulfate (590 μg/dL). Global ischemia with circulatory arrest ($n = 12$) was followed by transient 42- and 10-fold increases in syndecan-1 and heparan sulfate, respectively. After regional ischemia of heart and lungs (cardiopulmonary bypass; $n = 6$), syndecan-1 increased 65-fold, and heparan sulfate increased 19-fold. Infrarenal ischemia was followed by 15- and 3-fold increases, respectively ($n = 14$).	Rehm M, Bruegger D, Christ F, et al. Shedding of the endothelial glycocalyx in patients undergoing major vascular surgery with global and regional ischemia. Circulation. 2007;116(17):1896–1906. doi:10.1161/ CIRCULATIONAHA.106.684852
Syndecan-1	Coronary artery bypass surgery with and without cardiopulmonary bypass	44 undergoing coronary artery bypass grafting (22 on-pump vs. 22 off-pump)	Syndecan-1 increased from 29.5 ± 4.6 ng/mL at baseline to 98.7 ± 9.8 ng/mL	Svennevig K, Hoel T, Thiara A, et al. Syndecan-1 plasma levels during coronary artery bypass surgery with and without cardiopulmonary bypass. Perfusion. 2008;23 (3):165–171. doi:10.1177/0267659108098215
Syndecan-1	Coronary artery bypass surgery	15 on-pump conventional coronary artery bypass vs. 15 off-pump conventional coronary artery bypass	Conventional coronary artery bypass surgery led to significant median 2- and 4-fold increases in syndecan-1 and heparan sulfate concentrations, respectively. In patients undergoing OPCAB surgery, there were also significant increases in median syndecan-1 (4-fold) and heparan sulfate (2-fold). Syndecan-1 and heparan sulfate concentrations had returned to basal levels by the end of the operation in both groups.	Bruegger D, Rehm M, Abicht J, et al. Shedding of the endothelial glycocalyx during cardiac surgery: on-pump versus off-pump coronary artery bypass graft surgery. J Thorac Cardiovasc Surg. 2009;138 (6):1445–1447. doi:10.1016/j. jtcvs.2008.07.063
Syndecan-1, HS, HA	Coronary artery bypass surgery	Patients undergoing coronary artery bypass surgery with ($n = 15$) vs. without ($n = 15$) cardiopulmonary bypass at various phases of the procedure	Baseline levels: syndecan-1 (μg/g albumin), CCAB 2.9 (2.0/4.9), OPCAB 8.0 (2.7/14.4), heparan sulfate (μg/g albumin), CCAB 446.4 (199.3/744.0), OPCAB 1,003.5 (522.4/2,391.9), hyaluronan (μg/g albumin), CCAB 3.0 (2.0/5.7), OPCAB 3.4 (1.9/6.4). Maximum increases: syndecan-1 15-fold, heparan sulfate 9-fold, hyaluronan 5-fold basal.	Bruegger D, Schwartz L, Chappell D, et al. Release of atrial natriuretic peptide precedes shedding of the endothelial glycocalyx equally in patients undergoing on- and off-pump coronary artery bypass surgery. Basic Res Cardiol. 2011;106(6):1111–1121. doi:10.1007/s00395-011-0203-y
Syndecan-1, HA	Coronary artery disease undergoing coronary artery bypass grafting	13 patients (6 controls vs. 7 treated with diazoxide) with stable myocardial coronary artery disease eligible for elective CABG, with a history of a recent less than 1-month-old myocardial infarction	For time points 1, 2, and 3, respectively, syndecan-1 values for controls were 6.5 ± 1.0, 8.5 ± 2.2, and 5.6 ± 0.9 ng/mL, and for diazoxide-treated patients 3.5 ± 0.7, 7.6 ± 0.7, and 4.2 ± 0.6 ng/mL. Hyaluronan values for controls were 16.2 ± 6.6, 48.5 ± 13.3, and 56.1 ± 15.4 ng/mL, and for diazoxide-treated patients 18.3 ± 3.5, 117.2 ± 22.6, and 64.3 ± 7.9 ng/mL. Statistically,	Mennander AA, Shalaby A, Oksala N, et al. Diazoxide may protect endothelial glycocalyx integrity during coronary artery bypass grafting. Scand Cardiovasc J. 2012;46(6):339–344. doi:10.3109/14017431.2012.717303

Supplementary Table S1 (*Continued*)

Serum proteoglycans/ GAGs	CVD typology	Casuistry	Main findings	Reference
			neither the values of syndecan-1 nor hyaluronan differed at any time points 1, 2, or 3 between the patient groups.	
Perlecan LG3 fragment (C-terminal)	Acute and chronic vascular rejection (VR) of solid allografts	16 patients with acute vascular rejection (AVR), 16 with acute tubulointerstitial rejection (ATIR) vs. 32 stable graft function (controls)	Circulating LG3 levels were significantly higher in subjects with AVR compared with those with ATIR and normal allografts. Furthermore, AVR patients who lost their grafts in the first 6 months after transplantation ($n = 9$) had higher LG3 levels than patients with preserved allograft function for more than 6 months ($n = 7$). LG3 increased by 4.7 U for each 10 mL/min decrease in GFR.	Soulez M, Pilon EA, Dieudé M, et al. The perlecan fragment LG3 is a novel regulator of obliterative remodeling associated with allograft vascular rejection. Circ Res. 2012;110(1):94–104. doi:10.1161/CIRCRESAHA.111.250431
Syndecan-1	Thromboembolic events after Fontan procedure	76 randomly selected patients from 210 Fontan patients.	Median value of syndecan-1 was higher in fontan patients (46.4 ng/mL) compared with nonmatched reference value (17 ng/mL).	Idorn L, Jensen AS, Juul K, et al. Thromboembolic complications in Fontan patients: population-based prevalence and exploration of the etiology. Pediatr Cardiol. 2013;34(2):262–272. doi:10.1007/s00246-012-0431-4
Syndecan-1	Fontan circulation surgery (childhood)	34 Fontan circulation patients vs. 32 controls	Syndecan-1 level in patients: fontan without aspirin ($n = 13$) 510 [262–886] pg/mL, fontan with aspirin ($n = 12$) 544 [412–1038] pg/mL.	Tomkiewicz-Pajak L, Wojcik T, Chłopicki S, et al. Aspirin resistance in adult patients after Fontan surgery. Int J Cardiol. 2015;181:19–26. doi:10.1016/j.ijcard.2014.11.219
Syndecan-4	Open heart surgery	30 patients operated for severe, symptomatic aortic stenosis (AS) with aortic valve replacement (AVR)	Levels of shed syndecan-4 in blood collected from the LV increased 3-fold (4.80 ± 1.28 in LPS vs. 1.60 ± 0.49 ng/mL in controls). Levels of shed syndecan-4 in blood collected from the LV increased 3-fold (4.80 ± 1.28 in LPS vs. 1.60 ± 0.49 - ng/mL in controls). *Consistent with syndecan-4 being shed from the human heart, circulating syndecan-4 levels were 2.8-fold higher in the coronary sinus (14.95 ± 3.33 vs. 9.83 ± 2.51ng/mL in the radial artery)*.	Strand ME, Aronsen JM, Braathen B, et al. Shedding of syndecan-4 promotes immune cell recruitment and mitigates cardiac dysfunction after lipopolysaccharide challenge in mice. J Mol Cell Cardiol. 2015;88:133–144. doi:10.1016/j.yjmcc.2015.10.003
		Pre-LPS mice: WT $n = 8$, syn-4 KO $n = 8$; post-LPS WT $n = 13$, syn-4 KO $n = 13$; post-PBS WT $n = 12$, syn-4 KO $n = 12$.	9 hours after intraperitoneal injection of LPS (10 mg/kg) in WT mice, LV syndecan-4 mRNA was increased 9.8-fold compared with PBS-injected controls. Levels of shed syndecan-4 in blood increased 3-fold (4.80 ± 1.28 in LPS vs. 1.60 ± 0.49 ng/mL in controls)	
Syndecan-1, HA, HS	Cardiopulmonary bypass	30 patients undergoing cardiac surgery	Multifold increases in plasma syndecan-1, heparan sulfate, and HA concentrations of patients undergoing CPB. The median concentrations of syndecan-1 (4.8-fold) and HA (1.5-fold) at T4 significantly increased relative to those at T0. Heparan sulfate concentrations peaked at T5 (3.1-fold). HA and syndecan-1 concentrations returned to basal levels at T6 and T7, respectively. By contrast, heparan sulfate concentration increased slightly but failed to return to the baseline value and persisted after T7.	Wu Q, Gao W, Zhou J, et al. Correlation between acute degradation of the endothelial glycocalyx and microcirculation dysfunction during cardiopulmonary bypass in cardiac surgery. Microvasc Res. 2019;124:37–42. doi:10.1016/j.mvr.2019.02.004
Syndecan-1	Cardiopulmonary bypass	Blood samples from 30 patients (trial cohort—5 perioperative time points) vs. 30 patients (preoperatively)	In the trial cohort the increase of syndecan-1 was 2.7-fold. Plasma syndecan-1 correlated with all cytokines preoperatively.	Pesonen E, Passov A, Andersson S, et al. Glycocalyx degradation and inflammation in cardiac surgery. J Cardiothorac Vasc Anesth. 2019;33(2):341–345. doi:10.1053/j.jvca.2018.04.007

(*Continued*)

Supplementary Table S1 (*Continued*)

Serum proteoglycans/ GAGs	CVD typology	Casuistry	Main findings	Reference
Syndecan-1, HS	Cardiac surgery with cardiopulmonary bypass	17 patients who had undergone on-pump coronary artery bypass graft surgery (during the first 3 postoperative days).	Increased plasma heparan sulfate levels were inversely associated with the proportion of perfused vessels during cardiopulmonary bypass. Plasma syndecan-1 levels were inversely associated with the proportion of perfused vessels during the entire study period. The onset of bypass was associated with a 2-fold increase in plasma levels of heparan sulfate, followed by a 6-fold increase in plasma levels of syndecan-1 after weaning from bypass compared with preoperative values. Heparan sulfate levels were restored to prebypass levels after weaning from bypass, whereas syndecan-1 levels remained increased in the first 3 days following surgery.	Dekker NAM, Veerhoek D, Koning NJ, et al. Postoperative microcirculatory perfusion and endothelial glycocalyx shedding following cardiac surgery with cardiopulmonary bypass. Anesthesia. 2019;74 (5):609–618. doi:10.1111/ anae.14577
Syndecan-1	Proliferative vasculopathy systemic sclerosis	65 systemic sclerosis patients vs. 20 healthy individuals	Serum syndecan-1 levels were significantly higher in SSc patients than in healthy controls (76.8 vs. 50.3 ng/mL). Furthermore, serum syndecan-1 levels were higher in late-stage dcSSc patients (82.9 ng/mL), while not in non-late-stage dcSSc patients (69.5 ng/mL), than in healthy controls.	Wu CY, Asano Y, Taniguchi T, Sato S, Yu HS. Serum level of circulating syndecan-1: A possible association with proliferative vasculopathy in systemic sclerosis. J Dermatol. 2016;43(1):63–66. doi:10.1111/1346-8138.12986
Syndecan-1	Antiphospholipid syndrome	15 primary arterial APS patients vs. 15 healthy controls	Patients had significantly increased syndecan-1 plasma level (38.6 ± 5.0 vs. 19.1 ± 3.5 pg/mL) compared with control.	Miranda S, Billoir P, Le Besnerais M, et al. New insights into antiphospholipid-related endothelial dysfunction by assessment of vascular glycocalyx layer: results from a preliminary cross-sectional study. Lupus. 2020;29(2):157–164. doi:10.1177/0961203319897958
Heparan sulfate proteoglycan	**Pathology**	**Casuistry**	**Main findings**	**References**
Syndecan-1	Diabetes and obesity	17 type 1 diabetes mellitus with microalbuminuria vs. 12 normoalbuminuric type 1 diabetes mellitus	113 (89–143) vs. 39 (25–54) ($p < 0$ 0.0001) vs. 34 μg/mL (10–40 μg/mL) ($p < 0.00001$)	Svennevig K, Kolset SO, Bangstad HJ. Increased syndecan-1 in serum is related to early nephropathy in type 1 diabetes mellitus patients [published correction appears in Diabetologia. 2006 Dec;49 (12):3100. Dosage error in article text]. Diabetologia. 2006;49 (9):2214–2216. doi:10.1007/ s00125-006-0330-4
Glypican-4		Nondiabetic females ($n = 77$) and males ($n = 83$) grouped according to BMI and body fat distribution	Gpc4 is an insulin sensitizing "adipokine" that directly interacts with the insulin receptor to regulate its activation and downstream signaling; serum Gpc4 levels are positively correlated with body fat content and insulin resistance.	Ussar S, Bezy O, Blüher M, Kahn CR. Glypican-4 enhances insulin signaling via interaction with the insulin receptor and serves as a novel adipokine. Diabetes. 2012;61 (9):2289–2298. doi:10.2337/db11-1395
Glypican-4		76 nonalcoholic fatty liver disease (NAFLD) vs. 76 controls	Men vs. women: 1.83 (1.19–2.78) vs. 1.17 ng/mL (0.66–2.00 ng/mL) ($p < 0.001$); Gpc4 association with NAFLD and cardiometabolic risk factors including body fat distribution, insulin resistance, and arterial stiffness in women	Yoo HJ, Hwang SY, Cho GJ, et al. Association of glypican-4 with body fat distribution, insulin resistance, and nonalcoholic fatty liver disease. J Clin Endocrinol Metab. 2013;98 (7):2897–2901. doi:10.1210/ jc.2012-4297
Syndecan-1		42 T2DM vs. 31 healthy controls	26.15 ± 2.42 vs. 16.85 ± 1.98 ng/mL ($p = 0.005$); negative correlation with apoA1 ($r = -0.46$, $p = 0.003$)	Wang JB, Zhang YJ, Zhang Y, et al. Negative correlation between serum syndecan-1 and apolipoprotein A1 in patients with type 2 diabetes mellitus. Acta Diabetol. 2013;50 (2):111–115. doi:10.1007/s00592-010-0216-2

Supplementary Table S1 (*Continued*)

Serum proteoglycans/ GAGs	CVD typology	Casuistry	Main findings	Reference
Glypican-4		103 newly diagnosed T2DM vs. 92 Impaired glucose tolerance vs. 105 healthy controls	5.15 ± 1.20 vs. 7.17 ± 2.13 vs. 6.26 ± 2.21 ng/mL ($p < 0.01$); higher Gpc-4 levels in prediabetic subjects associated with insulin resistance and obesity	Li K, Xu X, Hu W, et al. Glypican-4 is increased in human subjects with impaired glucose tolerance and decreased in patients with newly diagnosed type 2 diabetes. Acta Diabetol. 2014;51(6):981–990. doi:10.1007/s00592-014-0652-5
Glypican-4		170 obese patients with different glucose metabolism status vs. 38 normal controls	Gpc4 levels elevated in obese patients with insulin resistance and positively correlated with BMI, systolic blood pressure (SBP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), fasting insulin (FINS), and homeostasis model assessment of insulin resistance (HOMA-IR)	Zhu HJ, Pan H, Cui Y, et al. The changes of serum glypican4 in obese patients with different glucose metabolism status. J Clin Endocrinol Metab. 2014;99(12): E2697–E2701. doi:10.1210/jc.2014-2018
Syndecan-1		100 type 2 diabetes mellitus with microalbuminuria vs. 100 type 2 diabetes mellitus without microalbuminuria vs. 50 controls	147.30 ± 80.27 vs. 59.38 ± 27.79 vs. 40.72 ± 1 9.11 ng/mL ($p < 0.001$); correlation with microalbuminuria ($r = 0.570$, $p < 0.001$)	Shet N, Shetty S, Rao AV. Syndecan-1 levels in type2 diabetes mellitus. IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) 2014, 13(2 ver II), 37–40
Syndecan-1		13 type 1 diabetes mellitus with kidney failure vs. 15 type 1 diabetes mellitus	Higher syndecan-1 levels in association with kidney failure ($p \leq 0.05$)	Kolseth IB, Reine TM, Parker K, et al. Increased levels of inflammatory mediators and proinflammatory monocytes in patients with type I diabetes mellitus and nephropathy. J Diabetes Complications. 2017;31 (1):245–252. doi:10.1016/j.jdiacomp.2016.06.029
Glypican-4		370 overweight and obese children aged 6–18 years	Correlation between GPC-4 and BMI; no correlation with insulin sensitivity and β-cell function indices	Leelalertlauw C, Korwutthikulrangsri M, Mahachoklertwattana P, et al. Serum glypican 4 level in obese children and its relation to degree of obesity. Clin Endocrinol (Oxf). 2017;87(6):689–695. doi:10.1111/cen.13435
Glypican-4		147 type 2 diabetes mellitus and 14 type 1 diabetes mellitus	Predictable factors of long-term diabetic complications	Cha JJ, Min HS, Kim K, et al. Long-term study of the association of adipokines and glucose variability with diabetic complications. Korean J Intern Med. 2018;33(2):367–382. doi:10.3904/kjim.2016.114
Heparan sulfate proteoglycan	**Pathology**	**Casuistry**	**Main findings**	**References**
Syndecan-1/ hyaluronidase/HA	Kidney disease	40 dialysis patients vs. 21 healthy controls	111.0 (79.8–154.0) vs. 27.5 (18.9–33.7 ng/mL) ($p < 0.01$); hyaluronidase activity: 28.2 (23.9–36.0) vs. 24.1 (20.9–26.9 U/mL) ($p = 0.01$); HA: 35.9 (14.5–70) vs. 16.8 (6.4–29.5 ng/mL) ($p = 0.02$)	Vlahu CA, Lemkes BA, Struijk DG, Koopman MG, Krediet RT, Vink H. Damage of the endothelial glycocalyx in dialysis patients. J Am Soc Nephrol. 2012;23(11):1900–1908. doi:10.1681/ASN.2011121181
Syndecan-1		23 ESRD patients vs. 10 patients who developed interstitial fibrosis/tubular atrophy after kidney transplantation vs. 12 patients with normal kidney function after successful living donor kidney transplantation vs. 10 healthy controls	Higher circulating levels in ESRD patients ($p < 0.01$); negative correlation with renal function (Spearman's $r = -0.67$, $p < 0.001$)	Dane MJ, Khairoun M, Lee DH, et al. Association of kidney function with changes in the endothelial surface layer. Clin J Am Soc Nephrol. 2014;9 (4):698–704. doi:10.2215/CJN.08160813
Syndecan-1/HA		95 chronic kidney disease patients vs. 31 healthy controls	4- and 2.2-fold higher levels of syndecan-1 and HA in CKD patients; inverse correlation among syndecan-1, HA, and estimated glomerular filtration rate, $r = -0.37$ ($p < 0.001$) and $r = -0.45$ ($p < 0.001$), respectively	Padberg JS, Wiesinger A, di Marco GS, et al. Damage of the endothelial glycocalyx in chronic kidney disease. Atherosclerosis. 2014;234 (2):335–343. doi:10.1016/j.atherosclerosis.2014.03.016

(*Continued*)

Supplementary Table S1 (*Continued*)

Serum proteoglycans/ GAGs	CVD typology	Casuistry	Main findings	Reference
Syndecan-1		510 renal transplant recipient (RTR) patients	Association among syndecan-1 and markers of renal and endothelial dysfunction	Adepu S, Rosman CW, Dam W, et al. Incipient renal transplant dysfunction associates with tubular syndecan-1 expression and shedding. Am J Physiol Renal Physiol. 2015;309 (2):F137–F145. doi:10.1152/ ajprenal.00127.2015
Syndecan-1		201 consecutive patients with acute decompensated heart failure: 62 acute kidney injury vs. 43 stable chronic kidney disease vs. 96 normal renal function	248.7 ± 165.6 vs. 98.1 ± 65.6 vs. 91.4 ± 42.9 ng/mL ($p < 0.001$); syndecan-1 associated with 6-month mortality rates	Neves FM, Meneses GC, Sousa NE, et al. Syndecan-1 in acute decompensated heart failure–association with renal function and mortality. Circ J. 2015;79(7):1511–1519. doi:10.1253/circj.CJ-14-1195
Syndecan-1		29 hemodialysis patients under respiratory muscle training (RMT, 8 weeks) vs. 12 hemodialysis patients	Significant reduction of syndecan-1 levels by RMT ($p < 0.05$)	Campos NG, Marizeiro DF, Florêncio ACL, et al. Effects of respiratory muscle training on endothelium and oxidative stress biomarkers in hemodialysis patients: A randomized clinical trial. Respir Med. 2018;134:103–109. doi:10.1016/j. rmed.2017.12.005
Syndecan-1		173 hemorrhagic fever with renal syndrome (HFRS) patients vs. 35 healthy controls	Higher syndecan-1 levels during the acute stage with respect to both the convalescent stage and the controls ($p < 0.05$)	Li J, Du H, Bai XF, et al. Study on expression of plasma sCD138 in patients with hemorrhagic fever with renal syndrome. BMC Infect Dis. 2018;18(1):100. Published 2018 Mar 1. doi:10.1186/s12879-018-3005-0
Syndecan-1/ HA/HS		15 postoperative patients vs. 15 healthy controls	Several-fold differences in the plasma concentration of shedding products from the endothelial glycocalyx layer (syndecan-1, HA, and HS) can be explained by variations in kidney function. Assumptions about shedding do not have to be made with less than a 5- to 6-fold change in plasma concentration	Hahn RG, Hasselgren E, Björne H, Zdolsek M, Zdolsek J. Biomarkers of endothelial injury in plasma are dependent on kidney function. Clin Hemorheol Microcirc. 2019;72 (2):161–168. doi:10.3233/CH-180444
Syndecan-1		84 hemodialysis patients (before and during dialysis)	Higher plasma syndecan-1 levels in men compared with women, $p < 0.0003$; patients in the highest syndecan-1 tertile had significantly less cardiovascular events and better survival compared with the lowest syndecan-1 tertile ($p = 0.02$ and $p = 0.005$, respectively)	Koch J, Idzerda NMA, Dam W, Assa S, Franssen CFM, van den Born J. Plasma syndecan-1 in hemodialysis patients associates with survival and lower markers of volume status. Am J Physiol Renal Physiol. 2019;316(1):F121–F127. doi:10.1152/ajprenal.00252.2018
Syndecan-1	Acute kidney injury after pediatric cardiac surgery	289 patients who underwent cardiac surgery	Median postoperative syndecan-1 levels were higher in patients with severe AKI (103.6 vs. 42.3). Postoperative plasma syndecan-1 is associated with subsequent severe acute kidney injury and poor outcomes among children undergoing cardiac surgery	de Melo Bezerra Cavalcante CT, Castelo Branco KM, Pinto Júnior VC, et al. Syndecan-1 improves severe acute kidney injury prediction after pediatric cardiac surgery. J Thorac Cardiovasc Surg. 2016;152 (1):178–186.e2. doi:10.1016/j. jtcvs.2016.03.079

Supplementary Table S2 Main findings of studies on the association of serum HS-PGs levels with various cancers, performed in the last 20 years

Heparan sulfate proteoglycan	Tumor typology	Casuistry	Main findings	References
Glypican-3	Hepatocellular carcinoma (HCC)	40 HCC vs. 13 liver cirrhosis vs. 34 chronic hepatitis vs. 60 healthy donors	GPC3 positivity in serum: 40.0% in HCC vs. 0% in the other groups	Nakatsura T, Yoshitake Y, Senju S, et al. Glypican-3, overexpressed specifically in human hepatocellular carcinoma, is a novel tumor marker. Biochem Biophys Res Commun. 2003;306 (1):16–25.
Glypican-3	HCC	34 HCC vs. 20 hepatitis plus liver cirrhosis vs. 18 hepatitis vs. 53 controls	GPC3 undetectable in both healthy individuals and patients with hepatitis; elevated GPC3 in 53% of patients with HCC; elevated GPC3 in 5% of patients with hepatitis plus cirrhosis	Capurro M, Wanless IR, Sherman M, et al. Glypican-3: a novel serum and histochemical marker for hepatocellular carcinoma. Gastroenterology. 2003;125(1):89–97. doi:10.1016/s0016-5085(03)00689-9
Glypican-3	HCC	69 patients with hepatocellular carcinoma vs. 38 patients with liver cirrhosis vs. 96 healthy controls	4.84 ± 8.91 ng/mL vs. 1.09 ± 0.74 - ng/mL ($p < 0.01$) vs. 0.65 ± 0.32 - ng/mL ($p < 0.001$)	Hippo Y, Watanabe K, Watanabe A, et al. Identification of soluble NH2-terminal fragment of glypican-3 as a serological marker for early-stage hepatocellular carcinoma. Cancer Res. 2004;64(7):2418–2423. doi:10.1158/0008-5472.can-03-2191
Glypican-3	HCC	50 HCC with cirrhosis vs. 41 cirrhosis	161.41 ± 422.33 ng/mL vs. 125.41 ± 281.05 ng/mL (not significant)	Beale G, Chattopadhyay D, Gray J, et al. AFP, PIVKAII, GP3, SCCA-1 and follisatin as surveillance biomarkers for hepatocellular cancer in non-alcoholic and alcoholic fatty liver disease. BMC Cancer. 2008;8:200. Published 2008 Jul 18. doi:10.1186/1471-2407-8-200
Glypican-3	HCC	200 HCC vs. 200 CLD	924.8 pg/mL (495.2–1335.6 pg/mL) vs. 1161.6 pg/mL (762.0–1784.0 pg/mL) ($p < 0.0001$); lower expression in HCC	Yasuda E, Kumada T, Toyoda H, et al. Evaluation for clinical utility of GPC3, measured by a commercially available ELISA kit with Glypican-3 (GPC3) antibody, as a serological and histological marker for hepatocellular carcinoma. Hepatol Res. 2010;40(5):477–485. doi:10.1111/j.1872-034X.2010.00624.x
Glypican-3	HCC	100 HCC vs. 50 intrahepatic cholangiocarcinoma vs. 50 patients with metastatic carcinoma vs. 50 liver cirrhosis vs. 50 chronic hepatitis vs. 40 healthy controls	Elevated serum GPC3 in 53% of HCC (ranging 35.5–7826.6 ng/mL) but undetectable in the other groups; serum GPC3 is highly specific for detecting HCC	Tangkijvanich P, Chanmee T, Komtong S, et al. Diagnostic role of serum glypican-3 in differentiating hepatocellular carcinoma from non-malignant chronic liver disease and other liver cancers. J Gastroenterol Hepatol. 2010;25(1):129–137. doi:10.1111/j.1440-1746.2009.05988.x
Glypican-3	HCC	Hepatocellular carcinoma vs. chronic liver disease	$\approx 800 \pm 200$ ng/mL vs. $\approx 350 \pm 50$ ng/mL ($p < 0.05$)	Suzuki M, Sugimoto K, Tanaka J, et al. Up-regulation of glypican-3 in human hepatocellular carcinoma. Anticancer Res. 2010;30(12):5055–5061
Glypican-3	HCC	37 HCC patients vs. 32 liver cirrhosis patients	GPC3 is a sensitive, specific serum and tissue marker for the diagnosis of early HCC	Liu H, Li P, Zhai Y, et al. Diagnostic value of glypican-3 in serum and liver for primary hepatocellular carcinoma. World J Gastroenterol. 2010;16 (35):4410–4415. doi:10.3748/wjg.v16.i35.4410
Glypican-3	HCC	36 HCC patients vs. 20 patients with secondary liver cancer vs. 25 patients with hepatitis B vs. 20 patients with hepatitis C vs. 28 patients with cirrhosis vs. 56 controls	116.8 ± 98.6 ng/mL vs. 24.60 ± 24.01 ng/mL vs. 13.67 ± 15.68 ng/mL vs. 6.73 ± 1.22 - ng/mL vs. 0.86 ± 1.12 ng/mL, $p < 0.05$	Zhang Q, Xiao Q, Lin Z, Ying X, Li Z, Lin JM. Development of a competitive radioimmunoassay for glypican-3 and the clinical application in diagnosis of hepatocellular carcinoma. Clin Biochem. 2010;43(12):1003–1008. doi:10.1016/j.clinbiochem.2010.04.074
Glypican-3	HCC	101 HC vs. 40 cirrhosis vs. 18 hepatitis vs. 30 controls	GPC-3 is a useful tumor marker complementary to AFP for clinical diagnosis of HCC	Qiao SS, Cui ZQ, Gong L, et al. Simultaneous measurements of serum AFP, GPC-3 and HCCR for diagnosing hepatocellular carcinoma. Hepatogastroenterology. 2011;58

(*Continued*)

Supplementary Table S2 (*Continued*)

Heparan sulfate proteoglycan	Tumor typology	Casuistry	Main findings	References
				(110–111):1718–1724. doi:10.5754/hge11124
Glypican-3	HCC	75 HCC vs. 55 cirrhosis vs. 28 healthy controls	5.13 pg/mL (53.9–93.2 pg/mL) vs. 5.51 pg/mL (53.9–236.2 pg/mL) vs. 3.9 pg/mL (53.9–7.7 pg/mL); diagnostic values: sensitivity 61.33%, specificity 41.82%, positive predictive value 58.97%, negative predictive values 44.43%; GPC3 not a useful diagnostic and prognostic marker for HCC	Ozkan H, Erdal H, Koçak E, et al. Diagnostic and prognostic role of serum glypican 3 in patients with hepatocellular carcinoma. J Clin Laboratory Anal. 2011;25(5):350–353. doi:10.1002/jcla.20484
Glypican-3	HCC	605 HCC vs. 25 controls	20.20 ± 5.41 μg/L vs. 1.92 ± 0.95 μg/L, $p < 0.01$	Li B, Liu H, Shang HW, Li P, Li N, Ding HG. Diagnostic value of glypican-3 in α fetoprotein negative hepatocellular carcinoma patients. Afr Health Sci. 2013;13(3):703–709. doi:10.4314/ahs.v13i3.26
Glypican-3	HCC	123 hepatocellular carcinoma vs. 70 liver cirrhosis vs. 70 chronic hepatitis vs. 56 acute hepatitis vs. 50 nonliver tumor vs. 30 healthy controls	Diagnostic values: sensitivity 52.8%, specificity 98.8%, diagnostic accuracy 83.5%, positive predictive value 95.6%, negative predictive value 80.7%	Yao M, Yao DF, Bian YZ, et al. Values of circulating GPC-3 mRNA and α-fetoprotein in detecting patients with hepatocellular carcinoma. Hepatobiliary Pancreat Dis Int. 2013;12(2):171–179. doi:10.1016/s1499-3872(13)60028-4
Glypican-3	HCC	40 newly diagnosed hepatocellular carcinomas vs. 10 patients with cirrhosis vs. 10 controls	Positivity: 95% vs. 10% vs. negative ($p < 0.001$); diagnostic values: sensitivity, 95%; specificity, 95%	Abdelgawad IA, Mossallam GI, Radwan NH, Elzawahry HM, Elhifnawy NM. Can Glypican3 be diagnostic for early hepatocellular carcinoma among Egyptian patients?. Asian Pac J Cancer Prev. 2013;14(12):7345–7349. doi:10.7314/apjcp.2013.14.12.7345
Glypican-3	HCC	155 HCC vs. 180 chronic hepatitis vs. 124 liver cirrhosis vs. 442 non-HCC cancer vs. 136 healthy controls	99.94 ± 267.2 ng/mL vs. 10.45 ± 46.02 ng/mL ($p < 0.0001$) vs. 19.44 ± 50.88 ng/mL ($p = 0.0013$) vs. 20.50 ± 98.33 ng/mL ($p < 0.0001$) vs. 4.14 ± 31.65 ng/mL ($p < 0.0001$); Application as a single marker in the diagnosis of HCC limited by sGPC3 presence in lung cancer and thyroid cancer	Chen M, Li G, Yan J, et al. Reevaluation of glypican-3 as a serological marker for hepatocellular carcinoma. Clin Chim Acta. 2013;423:105–111. doi:10.1016/j.cca.2013.04.026
Glypican-3	HCC	120 hepatocellular carcinomas (HCC) vs. 40 chronic liver disease (CLD)	75.8 ng/mL (21.7–482.5 ng/mL) vs. 66.4 ng/mL (2.33–66.4 ng/mL) ($p < 0.020$)	Lee HJ, Yeon JE, Suh SJ, et al. Clinical utility of plasma glypican-3 and osteopontin as biomarkers of hepatocellular carcinoma. Gut Liver. 2014;8(2):177–185. doi:10.5009/gnl.2014.8.2.177
Glypican-3	HCC	40 HCC vs. 10 cirrhosis vs. 10 healthy controls	7.7 ng/mL (4.9–11 ng/mL) vs. 2.74 - ng/mL (1.99–5.93 ng/mL) vs. 0.99 - ng/mL (0.86–1.67 ng/mL) ($p < 0.0001$); diagnostic values: sensitivity 95%, specificity 95%, diagnostic accuracy 95%, positive predictive value 97%, negative predictive value 90.5%	Abd El Gawad IA, Mossallam GI, Radwan NH, Elzawahry HM, Elhifnawy NM. Comparing prothrombin induced by vitamin K absence-II (PIVKA-II) with the oncofetal proteins glypican-3, Alpha feto protein and carcinoembryonic antigen in diagnosing hepatocellular carcinoma among Egyptian patients. J Egypt Natl Canc Inst. 2014;26(2):79–85. doi:10.1016/j.jnci.2014.01.001
Glypican-3	HCC	84 HCC vs. 80 cirrhosis vs. 32 hepatitis B vs. 61 healthy controls	GPC-3 tests negative in all 84 HCC patients	Wang Y, Yang H, Xu H, et al. Golgi protein 73, not Glypican-3, may be a tumor marker complementary to α-Fetoprotein for hepatocellular carcinoma diagnosis. J Gastroenterol Hepatol. 2014;29(3):597–602. doi:10.1111/jgh.12461
Glypican-3	HCC	30 HCC vs. 30 hepatitis C virus (HCV) cirrhosis vs. 20 healthy controls	551.47 ± 185.25 ng/mL vs. 98.23 ± 73.54 ng/mL ($p < 0.01$); diagnostic values: sensitivity 100%, specificity 93.3%, diagnostic accuracy 96.7%, positive predictive value 93.8%, negative predictive value 100%	Eman A.E. Badr, Tarek E. Korah, Ashraf Abdel Ghani, Sawsan El-Sayed & Safaa Badr (2014) Role of serum glypican-3 in the diagnosis and differentiation of small hepatocellular carcinoma from hepatitis-C virus cirrhosis, Alexandria

Supplementary Table S2 (*Continued*)

Heparan sulfate proteoglycan	Tumor typology	Casuistry	Main findings	References
				Journal of Medicine, 50:3:221–226, doi: 10.1016/j.ajme.2014.01.002
Glypican-3	HCC	157 newly diagnosed HCC vs. 156 patients with liver cirrhosis (LC)	0.80 ng/mL (0–3.09 ng/mL) vs. 0.60 - ng/mL (0.07–7.40 ng/mL) ($p = 0.255$)	Jeon Y, Jang ES, Choi YS, Kim JW, Jeong SH. Glypican-3 level assessed by the enzyme-linked immunosorbent assay is inferior to α-fetoprotein level for hepatocellular carcinoma diagnosis. Clin Mol Hepatol. 2016;22 (3):359–365. doi:10.3350/ cmh.2016.0033
Glypican-3	HCC	138 HCC vs. 56 liver cirrhosis vs. 62 liver fibrosis	Diagnostic power: sensitivity 93%, specificity 93%, positive predictive value 89%, negative predictive value 95%, efficiency 93%	Attallah AM, El-Far M, Omran MM, et al. GPC-HCC model: a combination of glybican-3 with other routine parameters improves the diagnostic efficacy in hepatocellular carcinoma. Tumour Biol. 2016;37(9):12571–12577. doi:10.1007/s13277-016-5127-6
Glypican-3	HCC	25 patients with stage I HCC who underwent surgical resection	33.7 ng/mL (7.5–1729.3 ng/mL) (postoperation) vs. 60.1 ng/mL (6.4–- 3466.8 ng/mL) ($p < 0.001$); postoperative recurrence associated with high preoperative plasma GPC3	Ofuji K, Saito K, Suzuki S, et al. Perioperative plasma glypican-3 level may enable prediction of the risk of recurrence after surgery in patients with stage I hepatocellular carcinoma. Oncotarget. 2017;8(23):37835–- 37844. doi:10.18632/ oncotarget.14271
Glypican-3	HCC	145 HCC vs. 105 chronic liver cirrhosis vs. 50 healthy controls	Diagnostic values: sensitivity 95%, specificity 100%, diagnostic accuracy 95%, positive predictive value 97.5%, negative predictive value 90.5%	Farag R. M. M. A, Al-Ayobi D, Alsaleh K. A, Kwon H. J, EL-Ansary A Dawoud E. A. Influence of glypican-3 as a newly diagnostic biomarker in early detection of hepatocellular carcinoma among saudi patients. Biomed Pharmacol J 2018;11(4)
Glypican-3	HCC	40 cirrhotic patients with primary HCC vs. 30 cirrhotic patients without HCC vs. 15 healthy controls	12.08 ± 14.11 ng/mL vs. 2.07 ± 1.44 - ng/mL vs. 1.25 ± 0.32 ng/mL ($p < 0.001$)	Tahon AM, El-Ghanam MZ, Zaky S, et al. Significance of glypican-3 in early detection of hepatocellular carcinoma in cirrhotic patients. J Gastrointest Cancer. 2019;50(3):434–441. doi:10.1007/s12029-018-0095-2
Glypican-3	HCC	25 HCC vs. 75 liver cirrhosis vs. 50 controls	Higher serum levels of GPC3 in HCC ($p < 0.001$); positive correlation between GPC3 and miR-1291	Hagag NA, Ali YBM, Elsharawy AA, Talaat RM. Clinical impact of circulated miR-1291 in plasma of patients with liver cirrhosis (LC) and hepatocellular carcinoma (HCC): implication on glypican-3 expression. J Gastrointest Cancer. 2020;51(1):234–241. doi:10.1007/s12029-019-00234-9
Glypican-3	HCC	25 hepatitis C virus-related HCC vs. 15 hepatitis B virus-related HCC vs. 16 HCC	9.9 pg/mL (2.8–273 pg/mL) vs. 2.6 pg/mL (0.5–384 pg/mL) vs. 3.0 pg/mL (0.5–22.1 pg/mL); association between GPC3 expression and secretion and the virus type	Shimizu Y, Mizuno S, Fujinami N, et al. Plasma and tumoral glypican-3 levels are correlated in patients with hepatitis C virus-related hepatocellular carcinoma. Cancer Sci. 2020;111 (2):334–342. doi:10.1111/cas.14251
Syndecan-1	Multiple myeloma (MM)	174 patients vs. 40 healthy controls	643 units/mL (401–2,022 units/mL) vs. 128 units/mL (76–208 units/mL), $p < 0.0001$; survival: 20 months vs. 44 months ($p < 0.0001$)	Seidel C, Sundan A, Hjorth M, et al. Serum syndecan-1: a new independent prognostic marker in multiple myeloma [published correction appears in Blood 2000 Apr 1;95(7):2197]. Blood. 2000;95(2):388–392
Syndecan-1	MM	25 newly diagnosed MM patients	Soluble Syndecan-1 higher in nonresponders to chemotherapy vs. responders ($p < 0.01$), and in nonsurvivors vs. survivors ($p < 0.001$); plasma cells' Syndecan-1 expression lower in nonresponders to chemotherapy vs. responders ($p < 0.01$), in nonsurvivors vs. survivors ($p < 0.05$)	Aref S, Goda T, El-Sherbiny M. Syndecan-1 in multiple myeloma: relationship to conventional prognostic factors. Hematology. 2003;8 (4):221–228. doi:10.1080/1024533031000153630
Syndecan-1	MM	67 newly diagnosed MM patients vs. 18 controls	$1,053.92 \pm 292.72$ ng/mL vs. 81.28 ± 8.83 ng/mL, $p < 0.0001$;	Andersen NF, Standal T, Nielsen JL, et al. Syndecan-1 and angiogenic

(*Continued*)

Supplementary Table S2 (*Continued*)

Heparan sulfate proteoglycan	Tumor typology	Casuistry	Main findings	References
			Syndecan-1 is a strong independent prognostic factor of shorter survival	cytokines in multiple myeloma: correlation with bone marrow angiogenesis and survival. Br J Haematol. 2005;128 (2):210–217. doi:10.1111/j.1365-2141.2004.05299.x
Syndecan-1	MM	27 MM patients vs. 11 healthy controls	177.5 ng/mL (34–3,500 ng/mL) vs. 40 ng/mL (28–75 ng/mL), $p = 0.001$; correlation with stage and shorter survival	Kyrtsonis MC, Vassilakopoulos TP, Siakantaris MP, et al. Serum syndecan-1, basic fibroblast growth factor and osteoprotegerin in myeloma patients at diagnosis and during the course of the disease. Eur J Haematol. 2004;72 (4):252–258. doi:10.1046/j.0902-4441.2003.00205.x
Syndecan-1	MM	501 patients	Survival: Syndecan-1 $>$ 158 ng/mL vs. Syndecan-1 $<$ 158 ng/mL, 36.3 months vs. 49.3 months ($p < 0.0001$)	Kumar S, Blood E, Oken MM, Greipp PR. Prognostic value of syndecan-1 in multiple myeloma and its relationship with other prognostic factors. Blood 2004, 104 (11): 2402
Syndecan-1	MM	13 patients with monoclonal gammopathy of undetermined significance vs. 4 patients with solitary plasmacytoma vs. 50 patients with MM; 6 months follow-up of MM patients receiving chemotherapy	77.9 ng/mL (33–122 ng/mL) vs. 65.6 - ng/mL (33.8–94.5 ng/mL) vs. 223.8 - ng/mL (36–508 ng/mL); Responders to chemotherapy ($n = 11$): 106 ng/mL (57.3–440 ng/mL) (after 6 months) vs. 258 ng/mL (97.3–460 ng/mL) (baseline), Nonresponders ($n = 4$): 361.7 - ng/mL (251–486 ng/mL) (after 6 months) vs. 327 ng/mL (245–466 ng/mL) (baseline)	Jánosi J, Sebestyén A, Mikala G, Németh J, Kiss Z, Vályi-Nagy I. Soluble syndecan-1 levels in different plasma cell dyscrasias and in different stages of multiple myeloma. Haematologica. 2004;89(3):370–371.
Syndecan-1	MM	324 patients at presentation vs. 154 patients at plateau phase	336 ng/mL (143–1635 ng/mL) vs. 192 ng/mL (111–422 ng/mL) ($p < 0.0006$)	Lovell R, Dunn JA, Begum G, et al. Soluble syndecan-1 level at diagnosis is an independent prognostic factor in multiple myeloma and the extent of fall from diagnosis to plateau predicts for overall survival. Br J Haematol. 2005;130(4):542–548. doi:10.1111/j.1365-2141.2005.05647.x
Syndecan-1	MM	17 MM patients vs. 14 patients with monoclonal gammopathy	1542 ng/mL (10–17,300 ng/mL) vs. 32 ng/mL (5–128 ng/mL), $p < 0.001$	Maisnar V, Tousková M, Tichý M, et al. The significance of soluble CD138 in diagnosis of monoclonal gammopathies. Neoplasma. 2006;53(1):26–29
Syndecan-1	MM	28 patients vs. 50 controls; 6 months follow-up of MM patients receiving chemotherapy	265 ng/mL (98–1,049 ng/mL) vs. 81 - ng/mL (27–192 ng/mL) ($p < 0.0001$); responders to chemotherapy (n: 20): 97 ng/mL (60–470 ng/mL)(after 6 months) vs. 220 ng/mL (98–1,049 ng/mL) (baseline), nonresponders (n: 8): 266 ng/mL (172–629 ng/mL) (after 6 months) vs. 337 ng/mL (265–785 ng/mL) (baseline)	Kim JM, Lee JA, Cho IS, Ihm CH. Soluble syndecan-1 at diagnosis and during follow up of multiple myeloma: a single institution study. Korean J Hematol. 2010;45(2):115–119. doi:10.5045/kjh.2010.45.2.115
Syndecan-1	MM	50 intact immunoglobulin MM vs. 34 light-chain MM vs. 40 healthy controls	90.73 ± 103.80 ng/mL vs. 40.21 ± 55.22 ng/mL vs. 15 ± 9.02 - ng/mL ($p = 0.012$; $p < 0.0001$; $p = 0.006$)	Cigliana G, Torti E, Gulli F, et al. Relationship between circulating syndecan-1 levels (CD138s) and serum free light chains in monoclonal gammopathies. J Exp Clin Cancer Res. 2015;34 (1):37. Published 2015 Apr 23. doi:10.1186/s13046-015-0155-4
Syndecan-1	Hodgkin's lymphoma	66 patients vs. 14 healthy controls	100.2 ± 35.9 ng/mL vs. 67.9 ± 24.5 - ng/mL ($p < 0.001$)	Vassilakopoulos TP, Kyrtsonis MC, Papadogiannis A, et al. Serum levels of soluble syndecan-1 in Hodgkin's lymphoma. Anticancer Res. 2005;25 (6C):4743–4746
Perlecan	Prostate cancer	288 prostate cancer patients vs. 12 healthy controls	Perlecan fragments associated with MMP-7 in prostate cancer tissues; domain IV perlecan in stage IV, but absent in normal sera. Perlecan fragments in sera and MMP-7 in tissues are measures of invasive prostate cancer	Grindel B, Li Q, Arnold R, et al. Perlecan/HSPG2 and matrilysin/MMP-7 as indices of tissue invasion: tissue localization and circulating perlecan fragments in a cohort of 288 radical prostatectomy patients [published correction appears in Oncotarget.

Supplementary Table S2 (*Continued*)

Heparan sulfate proteoglycan	Tumor typology	Casuistry	Main findings	References
				2016 Sep 13;7(37):60775]. Oncotarget. 2016;7(9):10433–10447. doi:10.18632/oncotarget.7197
Glypican-1	Prostate cancer	15 prostate cancer vs. 15 benign prostatic hyperplasia vs. controls	GPC-1 reduction in prostate cancer patients ($p < 0.05$)	Levin RA, Lund ME, Truong Q, et al. Development of a reliable assay to measure glypican-1 in plasma and serum reveals circulating glypican-1 as a novel prostate cancer biomarker. Oncotarget. 2018;9(32):22359-–22367. Published 2018 Apr 27. doi:10.18632/oncotarget.25009
Syndecan-1	Castration-resistant prostate cancer (CRPC)	75 patients who received docetaxel therapy until the appearance of therapy resistance	Serum SDC1 may help to facilitate clinical decision-making regarding the type and timing of therapy for patients with CRPC; positive correlation between SDC1 and MMP7	Szarvas T, Sevcenco S, Módos O, et al. Circulating syndecan-1 is associated with chemotherapy-resistance in castration-resistant prostate cancer. Urol Oncol. 2018;36(6):312.e9–312.e15. doi:10.1016/j.urolonc.2018.03.010
Exosomal glypican-1	Pancreatic ductal adenocarcinoma (PDAC)	3 Pancreatic ductal adenocarcinoma vs. 3 chronic pancreatitis vs. 6 healthy controls	Glypican-1 is not diagnostic for pancreatic ductal adenocarcinoma	Lai X, Wang M, McElyea SD, Sherman S, House M, Korc M. A microRNA signature in circulating exosomes is superior to exosomal glypican-1 levels for diagnosing pancreatic cancer. Cancer Lett. 2017;393:86–93. doi:10.1016/j.canlet.2017.02.019
Exosomal glypican-1	Breast cancer, pancreatic ductal adenocarcinoma	32 breast cancer vs. 190 pancreatic ductal adenocarcinoma vs. 100 healthy controls	Levels of GPC1+ circulating exosomes correlate with tumor burden and the survival of pre- and postsurgical patients	Melo SA, Luecke LB, Kahlert C, et al. Glypican-1 identifies cancer exosomes and detects early pancreatic cancer. Nature. 2015;523(7559):177–182. doi:10.1038/nature14581
Syndecan-1	Lung cancer	184 patients (138 non-small cell lung cancer and 46 small cell lung cancer)	Survival: Syndecan-1 > 59 ng/mL vs. Syndecan-1 < 59 ng/mL, 4 months vs. 11 months ($p < 0.0001$)	Joensuu H, Anttonen A, Eriksson M, et al. Soluble syndecan-1 and serum basic fibroblast growth factor are new prognostic factors in lung cancer. Cancer Res. 2002;62(18):5210–5217
Syndecan-1	Chronic lymphocytic leukemia	52 patients vs. 12 healthy controls	149.0 ng/mL (13.2–257.1 ng/mL) vs. 36.7 ng/mL (17.4–135.8 ng/mL)	Wołowiec D, Dybko J, Wróbel T, et al. Circulating sCD138 and some angiogenesis-involved cytokines help to anticipate the disease progression of early-stage B cell chronic lymphocytic leukemia. Mediators Inflamm. 2006;2006(3):42394. doi:10.1155/MI/2006/42394
Syndecan-1	Chronic lymphocytic leukemia	104 patients vs. 32 controls	52.8 ng/mL (13.4–252.7 ng/mL) vs. 19.86 ng/mL (14.49–33.14 ng/mL) ($p < 0.01$)	Jilani I, Wei C, Bekele BN, et al. Soluble syndecan-1 (sCD138) as a prognostic factor independent of mutation status in patients with chronic lymphocytic leukemia. Int J Laboratory Hematol. 2009;31(1):97–105. doi:10.1111/j.1751-553X.2007.01010.x
Exosomal glypican-1	Colorectal cancer	102 colorectal cancer vs. 80 healthy controls	Plasma GPC1+ exosomes, miR-96–5p, and miR-149 are specific markers for the diagnosis of CRC and targets for the therapy	Li J, Chen Y, Guo X, et al. GPC1 exosome and its regulatory miRNAs are specific markers for the detection and target therapy of colorectal cancer. J Cell Mol Med. 2017;21(5):838–847. doi:10.1111/jcmm.12941
Glypican-3	Hepatoblastoma	134 HB patients vs. 30 patients with benign hepatobiliary disorders vs. 20 controls	1.93 ng/mL (0–31.19) vs. 1.74 ng/mL (0–25.95) ($p = 0.6$) vs. 0.59 ng/mL (0–6.20) ($p = 0.003$); GPC3 is inferior to AFP as a serum marker for HB	Zhou S, O'Gorman MR, Yang F, Andresen K, Wang L. Glypican 3 as a Serum Marker for Hepatoblastoma. Sci Rep. 2017;7:45932. Published 2017 Apr 5. doi:10.1038/srep45932

Supplementary Table S3 Main findings of studies on the association of serum HS-PG levels with various trauma typologies, performed in the last 20 years

Serum proteoglycans/ GAGs	Trauma typology	Casuistry	Main findings	References
Syndecan-1	Trauma patients	75 patients	Median Syndecan-1 level 63 ng/mL (high glycocalyx degradation Syndecan-1 = 127 ng/mL, low glycocalyx degradation Syndecan-1 = 127 ng/mL)	Johansson PI, Stensballe J, Rasmussen LS, Ostrowski SR. A high admission syndecan-1 level, a marker of endothelial glycocalyx degradation, is associated with inflammation, protein C depletion, fibrinolysis, and increased mortality in trauma patients. Ann Surg. 2011;254(2):194–200. doi:10.1097/SLA.0b013e318226113d
Syndecan-1	Elderly trauma patients	80 adult trauma patients (38 higher age vs. 42 lower age)	Syndecan-1 levels: higher age 35 ng/mL, lower age 32 ng/mL	Johansson PI, Sørensen AM, Perner A, et al. Elderly trauma patients have high circulating noradrenaline levels but attenuated release of adrenaline, platelets, and leukocytes in response to increasing injury severity. Crit Care Med. 2012;40(6):1844–1850. doi:10.1097/CCM.0b013e31823e9d15
Syndecan-1	General trauma	80 trauma patients	sVEGFR1 correlated positively with Syndecan-1	Ostrowski SR, Sørensen AM, Windeløv NA, et al. High levels of soluble VEGF receptor 1 early after trauma are associated with shock, sympathoadrenal activation, glycocalyx degradation and inflammation in severely injured patients: a prospective study. Scand J Trauma Resusc Emerg Med. 2012;20:27. Published 2012 Apr 10. doi:10.1186/1757-7241-20-27
Syndecan-1	Trauma	80 trauma patients divided in 2 groups (high sCD40L and low sCD40L)	High sCD40L Syndecan-1 levels 42.9 ng/mL vs. low sCD40L Syndecan-1 levels 27.5 ng/mL.	Johansson PI, Sørensen AM, Perner A, et al. High sCD40L levels early after trauma are associated with enhanced shock, sympathoadrenal activation, tissue and endothelial damage, coagulopathy and mortality. J Thromb Haemost. 2012;10(2):207–216. doi:10.1111/j.1538-7836.2011.04589.x
Syndecan-1	Major trauma	88 patients	Significant association between cell free deoxyribonucleic acid levels and Syndecan-1 levels. 50 ng/mL change in Syndecan-1 corresponded to 15% increases in cfDNA levels	Naumann DN, Hazeldine J, Dinsdale RJ, et al. Endotheliopathy is associated with higher levels of cell-free DNA following major trauma: A prospective observational study. PLoS One. 2017;12(12):e0189870. Published 2017 Dec 19. doi:10.1371/journal.pone.0189870
Syndecan-1	Trauma	404 severely injured patients	Syndecan 1 levels 25 ng/mL.	Ostrowski SR, Henriksen HH, Stensballe J, et al. Sympathoadrenal activation and endotheliopathy are drivers of hypocoagulability and hyperfibrinolysis in trauma: A prospective observational study of 404 severely injured patients. J Trauma Acute Care Surg. 2017;82(2):293–301. doi:10.1097/TA.0000000000001304
Syndecan-1	Trauma	410 patients evaluated (endotheliopathy of trauma patients $n = 138$ vs. nonendotheliopathy of trauma patients $n = 272$)	Syndecn-1 levels: all patients 13 (26–-60) ng/mL, nonendotheliopathy of trauma patients 17 (10–26) ng/mL, endotheliopathy of trauma patients 108 (60–227) ng/mL.	Gonzalez Rodriguez E, Ostrowski SR, Cardenas JC, et al. Syndecan-1: a quantitative marker for the endotheliopathy of trauma. J Am Coll Surg. 2017;225(3):419–427. doi:10.1016/j.jamcollsurg.2017.05.012
Syndecan-1	Traumatic endotheliopathy	424 trauma patients	Syndecan-1 levels: 25 ng/mL (13–60)	Johansson PI, Henriksen HH, Stensballe J, et al. Traumatic endotheliopathy: a prospective observational study of 424 severely injured patients. Ann Surg. 2017;265(3):597–603. doi:10.1097/SLA.0000000000001751

Supplementary Table S3 (*Continued*)

Serum proteoglycans/ GAGs	Trauma typology	Casuistry	Main findings	References
Syndecan-1	Trauma and risk of sepsis	512 patients	Median Syndecan-1 levels at 4 hours after admission: 70 ng/dL (36–157 ng/dL) in patients who did not develop sepsis, and 165 ng/dL (67–336 ng/dL) in those who did.	Wei S, Gonzalez Rodriguez E, Chang R, et al. Elevated Syndecan-1 after trauma and risk of sepsis: a secondary analysis of patients from the Pragmatic, Randomized Optimal Platelet and Plasma Ratios (PROPPR) trial. J Am Coll Surg. 2018;227(6):587–595. doi:10.1016/j.jamcollsurg.2018.09.003
Syndecan-1	Endotheliopathy of trauma	12 patients with endotheliopathy of trauma (elevated Syndecan-1 levels) vs. 12 patients with lower levels	Patients with endotheliopathy of trauma had higher Syndecan-1, 230 (158–293) ng/mL vs. 19 (14–25) ng/mL	Wade CE, Matijevic N, Wang YW, et al. Absences of endothelial microvesicle changes in the presence of the endotheliopathy of trauma. Shock. 2019;51(2):180–184. doi:10.1097/SHK.0000000000001149
Syndecan-1	Shock-induced endotheliopathy (SHINE) in trauma	20 trauma patients vs. 20 healthy controls.	Patients with high circulating Syndecan-1 in plasma (244 [234–256] ng/mL) have an increased mortality rate compared with patients with lower levels (20 [10–25] ng/mL).	Henriksen HH, McGarrity S, Sigur&-ETH;ardóttir RS, et al. Metabolic systems analysis of shock-induced endotheliopathy (SHINE) in trauma: a new research paradigm [published online ahead of print, 2019 Jun 26]. Ann Surg. 2019;10.1097/SLA.0000000000003307. doi:10.1097/SLA.0000000000003307
Syndecan-1	Pediatric trauma	52 pediatric trauma patients vs. 12 control patients	Significant correlation between angiopoietin-2 and Syndecan-1	Richter RP, Russell RT, Hu PJ, et al. Plasma angiopoietin-2/-1 ratio is elevated and angiopoietin-2 levels correlate with plasma syndecan-1 following pediatric trauma. Shock. 2019;52(3):340–346. doi:10.1097/SHK.0000000000001267
Syndecan-1, HA, HS, CS	Severely injured trauma	5 healthy volunteers vs. 22 severely injured trauma patients	Patients vs. controls CS levels: 31.7 vs. 21.2 U/L; HS levels: 175.8 vs. 121.9 ng/mL; HA levels: 946.7 vs. 618.6 ng/mL; Syndecan-1 levels: 245.8 vs. 31.6 ng/mL.	Rahbar E, Cardenas JC, Baimukanova G, et al. Endothelial glycocalyx shedding and vascular permeability in severely injured trauma patients. J Transl Med. 2015;13:117. Published 2015 Apr 12. doi:10.1186/s12967-015-0481-5
Syndecan-1, HS	Polytrauma	30 patients vs. 6 healthy age- and sex-matched volunteers.	Syndecan-1 was strongly increased in patients with hemorrhagic shock. Levels of heparan sulfate were only slightly increased in plasma levels of hemorrhagic shock patients compared with those without hemorrhagic shock.	Halbgebauer R, Braun CK, Denk S, et al. Hemorrhagic shock drives glycocalyx, barrier and organ dysfunction early after polytrauma. J Crit Care. 2018;44:229–237. doi:10.1016/j.jcrc.2017.11.025
Syndecan-1	Trauma shock	80 adult trauma patients (12 acute coagulopathy of trauma shock positive vs. 68 normal)	Syndecan-1 levels: acute coagulopathy of trauma shock 62 ng/mL vs. normal 31 ng/mL.	Johansson PI, Sørensen AM, Perner A, et al. Disseminated intravascular coagulation or acute coagulopathy of trauma shock early after trauma? An observational study. Crit Care. 2011;15(6):R272. doi:10.1186/cc10553
Syndecan-1	Hemorrhagic shock and resuscitation	24 patients severely injured vs. 40 healthy controls	Injured patients in shock had markedly elevated plasma Syndecan-1 levels (554 ± 93 ng/mL) upon arrival. Levels significantly decreased with resuscitation (187 ± 36 ng/mL) but remained elevated above that of controls (27 ± 1 ng/mL). Postresuscitation Syndecan levels in patients who survived were 144 ± 141 ng/mL while in nonsurvivors were 289 ± 226 ng/mL.	Haywood-Watson RJ, Holcomb JB, Gonzalez EA, et al. Modulation of syndecan-1 shedding after hemorrhagic shock and resuscitation. PLoS One. 2011;6(8):e23530. doi:10.1371/journal.pone.0023530
Syndecan-1	Severe injury and early traumatic coagulopathy	77 trauma patients (4 patients displayed evidence of high-degree autoheparinization)	Patients with autoheparinization had 4-fold higher Syndecan-1 levels vs. nonheparinized (median 116 ng/mL vs. 31 ng/mL)	Ostrowski SR, Johansson PI. Endothelial glycocalyx degradation induces endogenous heparinization in patients with severe injury and early traumatic coagulopathy. J Trauma Acute Care Surg. 2012;73(1):60–66. doi:10.1097/TA.0b013e31825b5c10
Syndecan-1	Hemorrhagic shock	104 trauma patients, divided into low ($n = 34$) and normal ($n = 70$) colloid osmotic	Syndecan-1 levels were significantly higher (184 vs. 52 ng/mL) in patients with low colloid osmotic pressure	Rahbar E, Baer LA, Cotton BA, Holcomb JB, Wade CE. Plasma colloid osmotic pressure is an early indicator of injury

(*Continued*)

Supplementary Table S3 (*Continued*)

Serum proteoglycans/ GAGs	Trauma typology	Casuistry	Main findings	References
		pressure subgroups vs. 10 healthy control subjects.		and hemorrhagic shock. Shock. 2014;41(3):181–187. doi:10.1097/SHK.0000000000000101
Syndecan-1	Traumatic Brain Injury	80 patients sorted into 3 groups: isolated severe head/neck injuries ($n = 23$), severe head/neck and extracranial injuries ($n = 15$), and injuries without significant head/neck injuries ($n = 42$)	Patients Syndecan-1 levels: isolated severe head/neck injuries 33 (16–43) - ng/mL; severe head/neck and extracranial injuries: 59 (31–88) ng/mL; injuries without significant head/neck injuries: 31 (18–49) ng/mL	Genét GF, Johansson PI, Meyer MA, et al. Trauma-induced coagulopathy: standard coagulation tests, biomarkers of coagulopathy, and endothelial damage in patients with traumatic brain injury. J Neurotrauma. 2013;30 (4):301–306. doi:10.1089/neu.2012.2612
Syndecan-1	Traumatic brain injury	331 patients (68 with traumatic brain injury polytrauma, 58 with isolated traumatic brain injury, and 205 nontraumatic brain injury polytrauma).	Syndecan-1 levels: all patients 33.2 (16.6–79.1) ng/mL, nontraumatic brain injury polytrauma 31.5 (15.5–-61.2) ng/mL; polytraumatic brain injury 54.0 (25.1–107.0) ng/mL; isolated traumatic brain injury 25.0 (15.5–-44.4) ng/mL; controls 19.1 ng/mL	Gonzalez Rodriguez E, Cardenas JC, Cox CS, et al. Traumatic brain injury is associated with increased syndecan-1 shedding in severely injured patients. Scand J Trauma Resusc Emerg Med. 2018;26(1):102. Published 2018 Nov 21. doi:10.1186/s13049-018-0565-3

Supplementary Table S4 Main findings of studies on the association of serum HS-PG levels with sepsis, performed in the last 20 years

Serum proteoglycans/ GAGs	Pathology	Casuistry	Main findings	References
Syndecan-1		Experimental sepsis: DBA/2 mice challenged with *Bacillus anthracis* spores	Elevated levels of shed ectodomain readily detectable in circulation after 24 hours	Popova TG, Millis B, Bradburne C, et al. Acceleration of epithelial cell syndecan-1 shedding by anthrax hemolytic virulence factors. BMC Microbiol. 2006;6:8. Published 2006 Feb 7. doi:10.1186/1471-2180-6-8
Syndecan-1	Sepsis	127 nonpulmonary sepsis vs. 135 pulmonary sepsis	Higher levels of Syndecan-1 ($p = 0.017$); higher Syndecan-1 levels association with hepatic ($p < 0.001$), renal ($p = 0.003$), coagulation ($p = 0.001$), and circulatory ($p = 0.02$) failure as well as in-hospital mortality ($p = 0.001$)	Murphy LS, Wickersham N, McNeil JB, et al. Endothelial glycocalyx degradation is more severe in patients with non-pulmonary sepsis compared with pulmonary sepsis and associates with risk of ARDS and other organ dysfunction. Ann Intensive Care. 2017;7(1):102. Published 2017 Oct 6. doi:10.1186/s13613-017-0325-y
Syndecan-1	Sepsis	39 septic patients vs. 15 healthy controls	Higher levels of Syndecan-1 ($p < 0.0001$); association with disease severity, mortality and disseminated intravascular coagulation development	Ikeda M, Matsumoto H, Ogura H, et al. Circulating syndecan-1 predicts the development of disseminated intravascular coagulation in patients with sepsis. J Crit Care. 2018;43:48–53. doi:10.1016/j.jcrc.2017.07.049
Syndecan-1, HA	Sepsis	Experimental sepsis induced by endotoxin: 6 antithrombin-treated Wistar rats vs. 6 untreated controls	Syndecan-1: 31.75 ± 3.22 ng/mL vs. 42.62 ± 3.10 ng/mL ($p = 0.036$); HA: 155.6 ± 18.7 ng/mL vs. 256.2 ± 18.4 ng/mL ($p = 0.032$)	Iba T, Levy JH, Hirota T, et al. Protection of the endothelial glycocalyx by antithrombin in an endotoxin-induced rat model of sepsis. Thromb Res. 2018;171:1–6. doi:10.1016/j.thromres.2018.09.042
Glypican-1, -3, -4	Sepsis	184 patients with sepsis: 64 with organ failure vs. 120 without organ failure	GPC1: 28 ng/mL (18–36 ng/mL) vs. 19 ng/mL (14–26 ng/mL) ($p < 0.001$); GPC3: 2.8 ng/mL (1.9–3.7 ng/mL) vs. 1.7 ng/mL (1.3–2.3 ng/mL) ($p < 0.001$); GPC4: 4.3 ng/mL (2.6–7.8 ng/mL) vs. 1.7ng/mL (1.1–2.6 ng/mL) ($p < 0.001$); correlation with markers of disease severity, systemic inflammation, and glycocalyx damage	Fisher J, Linder A, Bentzer P. Elevated plasma glypicans are associated with organ failure in patients with infection. Intensive Care Med Exp. 2019;7(1):2. Published 2019 Jan 7. doi:10.1186/s40635-018-0216-z

Supplementary Table S4 (*Continued*)

Serum proteoglycans/ GAGs	Pathology	Casuistry	Main findings	References
Syndecan-1	Severe sepsis	184 patients with severe sepsis	Endothelial activation and damage may be linked to hypocoagulability in patients with severe sepsis	Ostrowski SR, Haase N, Müller RB, et al. Association between biomarkers of endothelial injury and hypocoagulability in patients with severe sepsis: a prospective study. Crit Care. 2015;19(1):191. Published 2015 Apr 24. doi:10.1186/s13054-015-0918-5
Syndecan-1	Severe sepsis	Cohort of patients with severe sepsis: intubated vs. not intubated; nonsurvivors vs. survivors; acute kidney injury vs. not acute kidney injury	No differences; 223 ng/mL (67–464 ng/mL) vs. 142 ng/mL (38–294 ng/mL) ($p = 0.04$); 193 ng/mL (63–441 ng/mL) vs. 93 ng/mL (23–187 ng/mL) ($p < 0.001$)	Puskarich MA, Cornelius DC, Tharp J, Nandi U, Jones AE. Plasma syndecan-1 levels identify a cohort of patients with severe sepsis at high risk for intubation after large-volume intravenous fluid resuscitation. J Crit Care. 2016;36:125–129. doi:10.1016/j.jcrc.2016.06.027
Syndecan-1	Severe sepsis	15 postthoracotomy patients with severe sepsis vs. 11 patients in recovery after open chest surgery (controls)	107.34 ± 84.79 ng/mL vs. 31.63 ± 19.93 ng/mL ($p = 0.008$)	Wu X, Hu Z, Yuan H, Chen L, Li Y, Zhao C. Fluid resuscitation and markers of glycocalyx degradation in severe sepsis. Open Med (Wars). 2017;12:409–416. Published 2017 Dec 22. doi:10.1515/med-2017-0059
Syndecan-1	Severe sepsis	20 patients with severe sepsis vs. 9 healthy male volunteers undergoing experimental endotoxemia	172 ± 102 ng/mL vs. 57 ± 42 ng/mL ($p < 0.05$)	Ostrowski SR, Berg RM, Windeløv NA, et al. Coagulopathy, catecholamines, and biomarkers of endothelial damage in experimental human endotoxemia and in patients with severe sepsis: a prospective study. J Crit Care. 2013;28(5):586–596. doi:10.1016/j.jcrc.2013.04.010
GAGs, Syndecan-1	Septic shock	18 septic shock patients vs. 18 healthy controls; nonsurvivors vs. survivors	GAGs levels: 2.7 µg/mL (1.9–4.8 µg/mL) vs. 1.8 µg/mL (1.7–2.0 µg/mL) ($p < 0.01$); 4.6 µg/mL (3.1–8.6 µg/mL) vs. 2.0 µg/mL (1.6–2.6 µg/mL) ($p < 0.01$). Syndecan-1: 246 ng/mL (180–496 ng/mL) vs. 26 ng/mL (23–31 ng/mL) ($p < 0.001$); no correlation with mortality	Nelson A, Berkestedt I, Schmidtchen A, Ljunggren L, Bodelsson M. Increased levels of glycosaminoglycans during septic shock: relation to mortality and the antibacterial actions of plasma. Shock. 2008;30(6):623–627. doi:10.1097/SHK.0b013e3181777da3
Syndecan-1, HS	Septic shock	104 patients with severe sepsis or septic shock vs. 28 patients after major abdominal surgery vs. 18 healthy volunteers	160 ± 109 ng/mL vs. 50.5 ± 46.9 - ng/mL vs. 20.5 ± 5.05 ng/mL ($p < 0.01$); 3.23 ± 2.43 µg/mL vs. 7.96 ± 3.26 µg/mL vs. 1.96 ± 1.21 µg/mL ($p < 0.05$)	Steppan J, Hofer S, Funke B, et al. Sepsis and major abdominal surgery lead to flaking of the endothelial glycocalix. J Surg Res. 2011;165(1):136–141. doi:10.1016/j.jss.2009.04.034
Syndecan-1	Septic shock	20 mechanically ventilated adult patients with septic shock vs. 20 healthy controls	Higher levels of syndecan-1 ($p < 0.001$)	Sallisalmi M, Tenhunen J, Yang R, Oksala N, Pettilä V. Vascular adhesion protein-1 and syndecan-1 in septic shock. Acta Anaesthesiol Scand. 2012;56(3):316–322. doi:10.1111/j.1399-6576.2011.02578.x
Syndecan-1	Septic shock	53 septic patients (shock at inclusion, 29) vs. 14 septic patients who received noradrenaline infusion (shock at inclusion, 10)	No differences ($p = 0.902$); shock at inclusion: 76 ng/mL (43–235 ng/mL) vs. 44 ng/mL (37–79) ($p = 0.048$)	Johansson PI, Haase N, Perner A, Ostrowski SR. Association between sympathoadrenal activation, fibrinolysis, and endothelial damage in septic patients: a prospective study. J Crit Care. 2014;29(3):327–333. doi:10.1016/j.jcrc.2013.10.028
HS, KS, HA, CS	Septic shock	24 septic shock patients vs. 24 controls	Both HS and HA increased in septic shock patients, particularly in patients that do not survive, and correlate with inflammatory activation and failing circulation	Nelson A, Berkestedt I, Bodelsson M. Circulating glycosaminoglycan species in septic shock. Acta Anaesthesiol Scand. 2014;58(1):36–43. doi:10.1111/aas.12223
Syndecan-1, HS	Septic shock	Septic shock model (dog)	Higher Syndecan-1 and HS levels in sepsis, correlated with inflammatory factors	Yini S, Heng Z, Xin A, Xiaochun M. Effect of unfractionated heparin on endothelial glycocalyx in a septic

(*Continued*)

Supplementary Table S4 (*Continued*)

Serum proteoglycans/ GAGs	Pathology	Casuistry	Main findings	References
				shock model. Acta Anaesthesiol Scand. 2015;59(2):160–169. doi:10.1111/aas.12418
Syndecan-1	Septic shock	13 septic shock vs. 100 severe sepsis vs. 95 sepsis vs. 63 local infection vs. 50 no infection	Progressive Syndecan-1 increases with increasing disease severity ($p < 0.001$)	Ostrowski SR, Gaïni S, Pedersen C, Johansson PI. Sympathoadrenal activation and endothelial damage in patients with varying degrees of acute infectious disease: an observational study. J Crit Care. 2015;30 (1):90–96. doi:10.1016/j.jcrc.2014.10.006
Syndecan-1, HS	Septic shock	56 septic shock patients vs. 100 sepsis patients vs. non-infected patients	Glycocalyx degradation, occurring in sepsis and septic shock, is associated with in-hospital mortality and the volume of intravenous fluids administered during sepsis resuscitation	Hippensteel JA, Uchimido R, Tyler PD, et al. Intravenous fluid resuscitation is associated with septic endothelial glycocalyx degradation. Crit Care. 2019;23(1):259. Published 2019 Jul 23. doi:10.1186/s13054-019-2534-2
Syndecan-1 and HS	Sepsis and major abdominal surgery	150 individuals with sepsis vs. major abdominal surgery	Syndecan-1 (ng/mL) controls: baseline 20.5 ± 5.05; 6 h 20.5 ± 5.05; 24 h 20.5 ± 5.05; 48 h 20.5 ± 5.05. Sepsis: baseline 160 ± 109; 6 h 141 ± 87.3; 24 h 161 ± 99.4; 48 h 165 ± 86.5. Surgery: baseline 50.5 ± 46.9; 6h 59.6 ± 59.8; 24 h 85.6 ± 131; 48 h 26.6 ± 16.9. Heparan sulfate (-mg/mL) controls: baseline 1.96 ± 1.21; 6 h 1.96 ± 1.21; 24 h 1.96 ± 1.21; 48 h 1.96 ± 1.21. Sepsis: baseline 3.23 ± 2.43; 6 h 4.99 ± 2.75; 24 h 5.68 ± 2.43; 48 h 3.45 ± 1.86. Surgery: baseline 7.96 ± 3.26; 6 h 8.48 ± 3.33; 24 h 8.49 ± 3.46; 48 h 6.77 ± 2.63.	Steppan J, Hofer S, Funke B, et al. Sepsis and major abdominal surgery lead to flaking of the endothelial glycocalix. J Surg Res. 2011;165(1):136–141. doi:10.1016/j.jss.2009.04.034
Endocan, Syndecan-1, HA	Pneumonia/sepsis	44 patients with sepsis due to pneumonia	Endocan decrease, Syndecan-1, and HA increase over time; Syndecan-1 association with neutrophil activation, the best EG biomarker predictor of adverse clinical outcomes	Smart L, Bosio E, Macdonald SPJ, et al. Glycocalyx biomarker syndecan-1 is a stronger predictor of respiratory failure in patients with sepsis due to pneumonia, compared with endocan. J Crit Care. 2018;47:93–98. doi:10.1016/j.jcrc.2018.06.015
Syndecan-1	Acute bacterial pneumonia	30 patients with acute bacterial pneumonia vs. 11 healthy controls	20.3 ± 8.9 ng/mL vs. 15.1 ± 2.6 -ng/mL ($p < 0.006$); negative correlation with the pneumonia severity score ($r = -0.391$, $p = 0.03$)	Nikaido T, Tanino Y, Wang X, et al. Serum Syndecan-4 as a possible biomarker in patients with acute pneumonia. J Infect Dis. 2015;212 (9):1500–1508. doi:10.1093/infdis/jiv234
Syndecan-1, -2, -3 and -4	Critical illness	137 patients with sepsis, cardiac arrest, gastrointestinal bleeding, intoxication or trauma vs. 6 surgical control patients before anesthesia and 6 healthy donors	Higher Syndecan-1 and -3; all Syndecans showed an association with mortality	Nelson A, Johansson J, Tydén J, Bodelsson M. Circulating syndecans during critical illness. APMIS. 2017;125(5):468–475. doi:10.1111/apm.12662
Syndecan-1	Malaria	12 patients who survived until day 14 vs. 20 healthy controls	Severe malaria is associated with endothelial activation that may contribute to microvascular thrombosis and endothelial damage	Graham SM, Chen J, Chung DW, et al. Endothelial activation, haemostasis and thrombosis biomarkers in Ugandan children with severe malaria participating in a clinical trial. Malar J. 2016;15:56. Published 2016 Feb 2. doi:10.1186/s12936-016-1106-z
Syndecan-1, HS, HA, CS	Dengue infection	103 dengue-infected patients	High Syndecan-1 and CS levels associated with plasma leakage	Suwarto S, Sasmono RT, Sinto R, Ibrahim E, Suryamin M. Association of endothelial glycocalyx and tight and adherens junctions with severity of plasma leakage in dengue infection. J Infect Dis. 2017;215 (6):992–999. doi:10.1093/infdis/jix041

Supplementary Table S5 Main findings of studies on the association of serum HS-PG levels with various pathophysiological conditions, performed in the last 20 years

Serum proteoglycans/ GAGs	Pathology	Casuistry	Main findings	References
Syndecan-1	Multicentric Castleman's disease	Case report	Marked elevation of soluble Syndecan-1, despite the absence of plasma cells, in bronchoalveolar lavage fluid in a patient with pulmonary involvement	Hasegawa M, Betsuyaku T, Yoshida N, et al. Increase in soluble CD138 in bronchoalveolar lavage fluid of multicentric Castleman's disease. Respirology. 2007;12(1):140–143. doi:10.1111/j.1440-1843.2006.00967.x
Syndecan-1	Crohn's disease (CD)	89 patients: 56 CD (19 remission, 37 active) vs. 18 with intestinal tuberculosis vs. 15 patients with functional bowel disorders	144.0 ± 8.15 ng/mL vs. 51.9 ± 4.95 ng/mL vs. 62.5 ± 4.37ng/mL ($p < 0.0001$); higher levels in active stage of CD	Zhang S, Qing Q, Wang Q, et al. Syndecan-1 and heparanase: potential markers for activity evaluation and differential diagnosis of Crohn's disease. Inflamm Bowel Dis. 2013;19(5):1025–1033. doi:10.1097/MIB.0b013e318280298f
Syndecan-1, -3, and -4, HA, CS, HS	Multiple sclerosis	Experimental autoimmune encephalitis in mice and rats	Increased CS and HA by 2- to 2.5-fold at the peak-stage of EAE whereas no change in HS; shedding of the GLX ectodomains of Syndecn-1, -3, and -4 seemed to be unaffected by inflammation, endothelial activation, and nervous tissue damage in the early phases studied	DellaValle B, Manresa-Arraut A, Hasseldam H, et al. Detection of glycan shedding in the blood: new class of multiple sclerosis biomarkers?. Front Immunol. 2018;9:1254. Published 2018 Jun 4. doi:10.3389/fimmu.2018.01254
Syndecan-1	Systemic lupus erythematosus (SLE)	22 SLE patients vs. 14 healthy controls	Correlation between levels and disease activity	Minowa K, Amano H, Nakano S, et al. Elevated serum level of circulating syndecan-1 (CD138) in active systemic lupus erythematosus. Autoimmunity. 2011;44(5):357–362. doi:10.3109/08916934.2010.545846
Syndecan-1	SLE	111 SLE patients vs. 18 patients with rheumatoid arthritis vs. 20 healthy subjects; 30 patients with active nephritis vs. 23 inactive nephritis vs. 58 without nephritis	34.2 ng/mL (20.9–54.0 ng/mL) vs. 12.8 ng/mL (8.7–21.5 ng/mL) vs. 18.5 ng/mL (14.5–27.6 ng/mL) ($p < 0.001$); 59.6 ng/mL (40.5–143.0 ng/mL) vs. 23.5 ng/mL (16.8–36.5 ng/mL) vs. 30.1 ng/mL (18.9–43.7 ng/mL) ($p < 0.001$)	Kim KJ, Kim JY, Baek IW, Kim WU, Cho CS. Elevated serum levels of syndecan-1 are associated with renal involvement in patients with systemic lupus erythematosus. J Rheumatol. 2015;42(2):202–209. doi:10.3899/jrheum.140568
HS, HA, CS	Respiratory failure in critically ill adults	7 direct lung injury vs. 6 indirect lung injury vs. 4 respiratory failure due to altered mental status vs. 4 healthy donors	Higher HS levels and sulfation in patients with indirect lung injury; higher HA in patients with direct lung injury; no differences in CS levels	Schmidt EP, Li G, Li L, et al. The circulating glycosaminoglycan signature of respiratory failure in critically ill adults. J Biol Chem. 2014;289(12):8194–8202. doi:10.1074/jbc.M113.539452
Syndecan-1 and -4	Chronic obstructive pulmonary disease (COPD)	101 COPD patients vs. 57 health controls	Downward trend in COPD patients ($p = 0.004$ and $p = 0.069$); positive correlation among Syndecan-1, the severity of airflow obstruction and CRP	Li D, Wu Y, Guo S, et al. Circulating syndecan-1 as a novel biomarker relates to lung function, systemic inflammation, and exacerbation in COPD. Int J Chron Obstruct Pulmon Dis. 2019;14:1933–1941. Published 2019 Aug 28. doi:10.2147/COPD.S207855
Syndecan-1	Lung cancer/lung resection surgery	16 patients before and after elective open lobectomy for primary lung cancer	1174 ± 335 pg/mL preoperative vs. 1797 ± 323 pg/mL postoperative ($p < 0.001$)	Arthur A, McCall PJ, Jolly L, Kinsella J, Kirk A, Shelley BG. Endothelial glycocalyx layer shedding following lung resection. Biomark Med. 2016;10(10):1033–1038. doi:10.2217/bmm-2016-0163
Syndecan-1	Inflammatory bowel disease	41 inflammatory bowel disease (22 Crohn's disease CD, 19 ulcerative colitis UC) vs. 16 healthy controls (HC)	29.5 ± 13.4 ng/mL vs. 21.1 ± 10.4ng/mL ($p = 0.03$); CD vs. HC, 32 ± 16.94 vs. 21.15 ± 10.39 ng/mL ($p = 0.01$); UC vs. HC, 26.7 ± 7.15 vs. 21.15 ± 10.39 ng/mL ($p = 0.18$); anti-inflammatory treatment reduces Syndecan-1 levels (26.45 ± 9.75 vs. 38 ± 18.43 ng/mL ($p = 0.008$)	Yablecovitch D, Stein A, Shabat-Simon M, et al. Soluble Syndecan-1 levels are elevated in patients with inflammatory bowel disease. Dig Dis Sci. 2015;60(8):2419–2426. doi:10.1007/s10620-015-3589-9

(*Continued*)

Supplementary Table S5 (*Continued*)

Serum proteoglycans/ GAGs	Pathology	Casuistry	Main findings	References
Syndecan-1	Ulcerative colitis	20 patients vs. 20 normal controls	Suppression of Syndecan-1 shedding by intestinal epithelial cells relieves severity of intestinal inflammation and neutrophil transmigration	Zhang Y, Wang Z, Liu J, et al. Cell surface-anchored syndecan-1 ameliorates intestinal inflammation and neutrophil transmigration in ulcerative colitis [published correction appears in J Cell Mol Med. 2017 Apr;21(4):834]. J Cell Mol Med. 2017;21(1):13–25. doi:10.1111/jcmm.12934
Syndecan-1	Knee arthroplasty	33 methylprednisolone treated vs. 30 controls	11.6 ± 1.0 ng/mL vs. 13.4 ± 1.1 - ng/mL ($p = 0.046$)	Lindberg-Larsen V, Ostrowski SR, Lindberg-Larsen M, Rovs.ing ML, Johansson PI, Kehlet H. The effect of pre-operative methylprednisolone on early endothelial damage after total knee arthroplasty: a randomized, double-blind, placebo-controlled trial. Anesthesia. 2017;72 (10):1217–1224. doi:10.1111/ anae.13983
Syndecan-1	Burn injury	Murine model	Burn injury causes shedding of Syndecan-1 correlated to injury severity and loss of the glycocalyx	Luker JN, Vigiola Cruz M, Carney BC, et al. Shedding of the endothelial glycocalyx is quantitatively proportional to burn injury severity. Ann Burns Fire Disasters. 2018;31 (1):17–22
Syndecan-1	End-stage liver disease	30 liver transplant recipients vs. 10 healthy volunteers	74.3 ± 59.9 ng/mL vs. 10.7 ± 9.4ng/mL ($p < 0.05$)	Schiefer J, Lebherz-Eichinger D, Erdoes G, et al. Alterations of endothelial glycocalyx during orthotopic liver transplantation in patients with end-stage liver disease. Transplantation. 2015;99 (10):2118–2123. doi:10.1097/ TP.0000000000000680
Syndecan-1	Critically ill patients receiving a prophylactic transfusion prior to an invasive procedure	33 patients before and after transfusion	565.1 pg/mL (126.8–1175.7 pg/mL) vs. 674.6 pg/mL (132.2–1689.8 pg/mL) ($p < 0.01$)	Straat M, Müller MC, Meijers JC, et al. Effect of transfusion of fresh frozen plasma on parameters of endothelial condition and inflammatory status in non-bleeding critically ill patients: a prospective substudy of a randomized trial. Crit Care. 2015;19(1):163. Published 2015 Apr 15. doi:10.1186/s13054-015-0828-6
Syndecan-1 and HS	Hemorrhage/ resuscitation strategies	9 rats were bled 40% of total blood volume and resuscitated with seven different fluids	Syndecan-1 and HS as valuable clinical biomarkers of glycocalyx shedding useful in guiding resuscitation strategies following hemorrhage	Torres Filho IP, Torres LN, Salgado C, Dubick MA. Plasma syndecan-1 and heparan sulfate correlate with microvascular glycocalyx degradation in hemorrhaged rats after different resuscitation fluids. Am J Physiol Heart Circ Physiol. 2016;310(11): H1468–H1478. doi:10.1152/ ajpheart.00006.2016
Syndecan-1	Chronic hypertensive pregnancy	25 pre-eclampsia vs. 92 chronic hypertension; 63 black ethnicity vs. 54 non-black ethnicity	Syndecan-1 concentrations increased across gestation in all subgroups ($p < 0.0001$) and decreased postpartum (-83%, $p < 0.001$). No differences were found in Syndecan-1 concentrations among subgroups	Webster LM, Gill C, Seed PT, et al. Chronic hypertension in pregnancy: impact of ethnicity and superimposed preeclampsia on placental, endothelial, and renal biomarkers. Am J Physiol Regul Integr Comp Physiol. 2018;315(1):R36–R47. doi:10.1152/ajpregu.00139.2017
Syndecan-1, HS, HA	Ovulatory cycle	16 healthy women (3 phases: early follicular phase, at ovulation, and mid-luteal phase)	Both Syndecan-1 and HS increased from phase 2 to phase 3 ($p = 0.031$, $p = 0.011$). No variation of HA during the ovulatory cycle	Hulde N, Rogenhofer N, Brettner F, et al. The CYCLOCALYX study: Ovulatory cycle affects circulating compartments of the endothelial glycocalyx in blood. Am J Reprod Immunol. 2018;79(1):10.1111/ aji.12767. doi:10.1111/aji.12767
HSPG, HA, Syndecan-1	Gestational diabetes mellitus/pre-eclampsia	43 normotensive vs. 10 gestational diabetes mellitus vs.		Weissgerber TL, Garcia-Valencia O, Milic NM, et al. Early onset

Supplementary Table S5 (*Continued*)

Serum proteoglycans/ GAGs	Pathology	Casuistry	Main findings	References
		11 late-onset pre-eclampsia vs. 11 early-onset pre-eclampsia	High HSPG and HA in early onset pre-eclampsia compared with normotensive pregnancy	preeclampsia is associated with glycocalyx degradation and reduced microvascular perfusion. J Am Heart Assoc. 2019;8(4): e010647. doi:10.1161/ JAHA.118.010647
Syndecan-1	Preeclampsia	37 individuals	Maternal plasma concentrations of shed (soluble) Sdc1 rise ~50-fold with gestation and revert postpartum. On average, women who later develop pre-eclampsia($n = 9$) have lower levels of soluble Sdc1 in maternal plasma at 20 weeks' gestation (before clinical disease onset) compared with women with uncomplicated pregnancy ($n = 19$) or gestational hypertension ($n = 9$). There was no difference in concentration of soluble Syndecan-1 postpregnancy between women with prior preeclampsia and prior uncomplicated pregnancies.	Gandley RE, Althouse A, Jeyabalan A, et al. Low soluble syndecan-1 precedes preeclampsia. PLoS One. 2016;11(6):e0157608. Published 2016 Jun 14. doi:10.1371/journal. pone.0157608
Syndecan-1, HS, HA	Abdominal hysterectomy	7 patients	No clear evidence for shedding of the endothelial glycocalyx layer	Nemme J, Hahn RG, Krizhanovs.kii C, Ntika S, Sabelnikovs. O, Vanags I. Minimal shedding of the glycocalyx layer during abdominal hysterectomy. BMC Anesthesiol. 2017;17 (1):107. Published 2017 Aug 22. doi:10.1186/s12871-017-0391-6
	Training			
Syndecan-1, HS, HA	Strenuous physical exercise	21 young, untrained healthy men performed a maximal incremental cycling exercise until exhaustion	No changes in serum Syndecan-1 and HS; HA reduction ($p = 0.0003$)	Majerczak J, Duda K, Chlopicki S, et al. Endothelial glycocalyx integrity is preserved in young, healthy men during a single bout of strenuous physical exercise. Physiol Res. 2016;65(2):281–291. doi:10.33549/physiolres.933049
Syndecan-1, HS	20 weeks of moderate-intensity endurance training	11 healthy young, untrained men	Lowering of glycocalyx shedding accompanied by an attenuation of oxidative stress	Majerczak J, Grandys M, Duda K, et al. Moderate-intensity endurance training improves endothelial glycocalyx layer integrity in healthy young men. Exp Physiol. 2017;102 (1):70–85. doi:10.1113/EP085887

Author Query Form (STH/02847)

Special Instructions: Author please write responses to queries directly on proofs and then return back.

Q1: AU: Please confirm that given names (red), middle names (black) and surnames (green) have been identified correctly. Author names in bibliographic citations and online will appear as: Lepedda AJ, Nieddu G, Piperigkou Z, Kyriakopoulou K, Karamanos N, Formato M. Circulating Heparan Sulfate Proteoglycans as Biomarkers in Health and Disease. Please confirm if this is correct.

Q2: AU: Please provide degrees for the authors “Konstantina Kyriakopoulou” and “Marilena Formato.”

Q3: AU: Please provide degree(s) for the corresponding author.

Q4: AU: There are color figures in the article. Please note that the cost of printing your figures in color is 845 euros for the 1st color image followed by 179 euros for each subsequent image (tax included). Please confirm if you agree to this cost, or confirm that it is OK to convert the images to black and white for printing. In case you choose to go with black and white images in print, please amend the color indicators, if any, appropriately. Note that the figures shall be published online in color free of cost.

Q5: AU: Please provide complete reference details of the sources from where figures 1 and 4 have been derived. Also, please confirm whether you have obtained permissions from the copyright holders of the 2 figures to use them in your article. If yes, please share a copy of the permission letter obtained for record-keeping purpose.

Q6: AU: Please check the data for all tables for accuracy as the tables have been converted from excel to word for processing.

Q7: AU: Please note, tables have been renumbered to maintain citation order.

Q8: AU: Please check and confirm all the tables as set for accuracy.

Q9: AU: Ref. 116 has been updated as per PubMed database. Please check.