Article

Factors enhancing serum syndecan-1 concentrations: a large-scale comprehensive medical examination

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Abstract: Endothelial disorders are related to various diseases. An initial endothelial injury is characterized by endothelial glycocalyx injury. We aimed to evaluate endothelial glycocalyx injury by measuring serum syndecan-1 concentrations in patients during comprehensive medical examinations. A single-center, prospective, observational study was conducted at Asahi University Hospital. The participants enrolled in this study were 1313 patients who underwent comprehensive medical examinations at Asahi University Hospital from January 2018, to June 2018. One patient undergoing hemodialysis was excluded from the study. At enrollment, blood samples were obtained, and study personnel collected demographic and clinical data. No treatments or exposures were conducted except for standard medical examinations and blood sample collection. Laboratory data were obtained by collection of blood samples at the time of study enrolment. According to nonlinear regression, the concentrations of serum syndecan-1 were significantly related to age ($p = 0.016$), aspartic aminotransferase concentration (AST, $p = 0.020$), blood urea nitrogen concentration (BUN, $p = 0.013$), triglyceride concentration ($p < 0.001$), and hematocrit ($p = 0.006$). These relationships were independent associations. Endothelial glycocalyx injury, which is reflected by serum syndecan-1 concentrations, is related to age, hematocrit, AST concentration, BUN concentration, and triglyceride concentration.

Keywords: Endothelial disorders; glycocalyx injury; syndecan-1; nonlinear regression.

1. Introduction

Endothelial disorders are closely related to many diseases, including diabetes mellitus [1], hypertension [2], hypercholesterolemia [3], tumorigenesis [4], ischemia/reperfusion injury [5], respiratory disorder [6], renal dysfunction [7], and autoimmune vasculitis [8]. Previous studies have suggested that treatments used for endothelial disorders could also prevent cardiovascular disease [8 9]. Vascular endothelial disorder exists prior to atherosclerosis, and flow-mediated dilation (FMD) after experimentally imposed increases in shear stress can be used as an index of endothelial function [10 11]. However, FMD is not applicable as a screening approach because it requires the use of echography for diagnosis. Although high-sensitivity C-reactive protein (CRP), lipoprotein-associated

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phospholipase A2, and pentraxin 3 have been used as biomarkers of endothelial disorder, these markers can only estimate the presence of unstable plaque and do not reflect early vascular endothelial lesions. To date, no biomarkers have been developed to detect early vascular endothelial lesions.

The endothelium exists on the inner surface of blood vessels as a thin monolayer and therefore is exposed to the circulating blood. Endothelial cells in direct contact with circulating blood are called vascular endothelial cells. Vascular endothelial cells line the entire circulatory system, from the heart to the smallest capillaries. All healthy endothelium is coated by the sugar-protein glycocalyx [12-17], which plays key roles in vascular homeostasis, including regulation of microvascular tone and endothelial permeability, maintenance of an oncotic gradient across the endothelial barrier, regulation of the adhesion and migration of leukocytes, and inhibition of intravascular thrombosis [18-20]. The glycocalyx is composed of cell-bound proteoglycans, glycosaminoglycan side chains, and sialoproteins [21-23]. Proteoglycans consist of a core protein, such as a syndecan family protein, to which glycosaminoglycan is linked. Syndecan-1 is the core protein in heparan sulfate proteoglycan, which is also found in the glycocalyx. Syndecan-1 is released from the endothelium upon injury to the glycocalyx, causing its concentration in the circulation to increase [24]. In fact, serum syndecan-1 was used as an endothelial injury marker in recent clinical studies of sepsis [25 26].

Therefore, in this study, we investigated risk factors for endothelial disorders according to serum syndecan-1 concentrations measured during comprehensive medical examinations.

2. Experimental Section

2.1. Study population

In total, 1313 patients who had comprehensive medical examinations at Asahi University Hospital from January 1st 2018, to June 30th 2018, participated in this study.

2.2 Ethics approval and consent to participate

Ethical permission was obtained from the medical ethics committee of Gifu University Graduate School of Medicine, Gifu, Japan (record no.: 29-214) and Asahi University, Mizuho, Japan (record no.: 30-29), and all patients provided written informed consent.

2.2 Consent for publication

Written informed consent was obtained from the patient for publication of this report.

2.3 Clinical assessments

At enrolment, blood samples were obtained, and study personnel collected demographic and clinical data. Body mass index (BMI) was calculated as weight (kg) / height (m)2. Medical and medication history were obtained from all patients. Patients who received hemodialysis were excluded from the analysis.

2.4. Laboratory data

Laboratory data were obtained by the collection of blood samples at the time of study enrollment more than 12 h after fasting. Serum syndecan-1 concentrations were measured using an enzyme-linked immunosorbent assay (950.640.192; Diaclone, Besancon, Cedex, France).

2.5. Statistical analyses

Descriptive statistics were presented as frequencies and percentages for categorical variables or as medians with interquartile ranges (IQRs) for continuous variables. The primary outcome was

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serum syndecan-1 concentration. Multivariable regression models were used to assess independent associations between serum syndecan-1 concentrations and blood parameters with adjustment for patient characteristics. Serum syndecan-1 concentrations were natural log-transformed to provide normality in the regression residuals. Beta-coefficients of the regression model were back-transformed to represent the percent increase in serum syndecan-1 concentration with a 50% increase in the corresponding covariate. Nonlinear associations between continuous variables and serum syndecan-1 concentrations were assessed by including nonlinear cubic splines in the regression model. A priori, we chose to include age, sex, BMI, systolic blood pressure, serum total protein, albumin, total bilirubin, aspartic aminotransferase concentration (AST), alanine transaminase, lactate dehydrogenase, blood urea nitrogen (BUN), creatinine concentration, CRP, fasting blood sugar concentration, hemoglobin a1c, serum triglyceride, high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol, uric acid concentration, hemoglobin concentration, hematocrit (Ht), white blood cell number, and platelet number in the regression model. To avoid the bias of the results by excluding missing data, we used multiple imputations in the regression model.

All analyses used a two-sided 5% significance level. Data management and analyses were performed using R version 3.5.1.

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3. Results

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation as well as the experimental conclusions that can be drawn.

3.1. Characteristics of the patients

Between January and June 2018, we enrolled 1313 patients. One patient undergoing hemodialysis was excluded from the study; thus, we included 1312 patients, with a median age of 51 years (Table 1), in this study. The patients were being treated for hypertension (n = 234, 17.8%), hyperlipidemia (n = 173, 13.2%), diabetes mellitus (n = 80, 6.1%), and hyperuricemia (n = 70, 5.3%). Malignant neoplasms were observed in 65 patients (5.0%; Table 1). Additionally, 85 patients were receiving no treatments and no abnormal laboratory data.

Table 1: Characteristics of the patients

	Median or Number	(25-75 Percentile)
Number of Cases	1312	
Age	51	(43-59)
Sex (M/F)	819/493	
BMI (kg/m^2)	22.6	(20.6-24.8)
SBP (mmHg)	122	(111-132)
TP (g/dL)	7.3	(7.1-7.6)
Alb (g/dL)	4.3	(4.2-4.5)
T-Bil (mg/dL)	0.7	(0.5-0.9)
AST (U/L)	17	(14-22)
ALT (U/L)	16	(12-23)
LDH (U/L)	242	(219-268)
BUN (mg/dL)	13.4	(11.4-15.8)
Cre (mg/dL)	0.77	(0.64-0.88)
CRP (mg/dL)	0.04	(0.02-0.09)
FBS (mg/dL)	97	(92-104)
HbA1c (%)	5.4	(5.3-5.6)
TG (mg/dL)	68	(47-99)
HDL –cho (mg/dL)	63	(51-78)
LDL-cho (mg/dL)	115	(97-134)
UA (mg/dL)	5.0	(4.0-6.0)
Hb (g/dL)	14.6	(13.4-15.5)
Ht (%)	42.1	(39.0-44.6)
WBC ($\times 10^3$/μl)	5000	(4200-5900)
Plt ($\times 10^4$/μl)	22.4	(19.3-25.7)
History of Present Illness	**Number**	**(Percentage)**
Hypertension	234	(17.8%)
Diabetes Mellitus	80	(6.1%)
Hyperlipidemia	173	(13.2%)
Hyper Uric Acid	70	(5.3%)
Malignant Neoplasm	65	(5.0%)

BMI: Body mass index, SBP: Systolic Blood Pressure, TP: Total protein, Alb: Albumin, T-Bil: Total bilirubin, AST: Aspartic Aminotransferase, ALT: Alanine Transaminase, LDH: Lactate Dehydrogenase, BUN: Blood Urea Nitrogen, Cre: Creatinine, CRP: C-reactive protein, FBS: Fasting Blood Sugar, HbA1c: Hemoglobin A1c, TG: Triglyceride, HDL-Cho: Heavy Density Lipoprotein-cholesterol, LDL-Cho: Low Density Lipoprotein-cholesterol, UA: Uric Acid, Hb; Hemoglobin, Ht: Hematocrit, WBC: White Blood Cell, Plt: Platelet

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3.2. Associations of serum syndecan-1 with various parameters

The results of multivariable regression analysis are shown in Table 2. Age, AST concentration, BUN concentration, triglyceride concentration, and Ht were significantly associated with serum syndecan-1 concentrations after adjustment for sex, medication and BMI. A 1 IQR increase in age was independently associated with a 0.9-fold increase in serum syndecan-1 (β = 0.903; 95% confidence interval [CI]: 0.831–0.982; p = 0.016; Fig 1A). Similar results were found for AST (β = 1.093; 95% CI: 0.996–1.200; p = 0.020; Fig 1B), BUN (β = 1.083; 95% CI: 1.018–1.152; p = 0.013; Fig 1C), triglyceride (β = 1.131; 95% CI: 1.030–1.242; p < 0.001; Fig 1D) and Ht (β = 1.726; 95% CI: 1.233–2.417; p = 0.006; Fig 1E) after adjustment for covariates. No significant associations were observed for other factors.

According to nonlinear regression analysis, serum syndecan-1 concentrations were significantly related to age, AST concentration, BUN concentration, triglyceride concentration, and Ht. These relationships were independent associations. Significant nonlinear associations were not observed for each variable.

Table 2: Results of multivariable regression analysis

	25 percentile	75 percentile	Fold-change [IQR]	95%LCI	95%UCI	p-value
Age	43	59	0.903	0.831	0.982	0.016
Sex – Female : Male	-	-	0.883	0.759	1.026	0.105
Medication – Yes : No	-	-	1.030	0.944	1.124	0.505
BMI (kg/m²)	20.6	24.8	0.995	0.939	1.054	0.863
SBP (mmHg)	111	132	0.972	0.908	1.039	0.471
TP (g/dL)	7.1	7.6	0.979	0.918	1.044	0.755
Alb (g/dL)	4.2	4.5	0.980	0.913	1.051	0.784
T-Bil (mg/dL)	0.5	0.9	0.940	0.865	1.021	0.168
AST (U/L)	14	22	1.093	0.996	1.200	0.020
ALT (U/L)	12	23	1.046	0.945	1.158	0.484
LDH (U/L)	219	268	0.979	0.916	1.045	0.680
BUN (mg/dL)	11.4	15.8	1.083	1.018	1.152	0.013
Cre (mg/dL)	0.64	0.88	1.048	0.952	1.154	0.591
CRP (mg/dL)	0.02	0.09	1.023	0.935	1.119	0.725
FBS (mg/dL)	92	104	0.993	0.929	1.061	0.603
HbA1c (%)	5.3	5.6	0.954	0.907	1.004	0.077
TG (mg/dL)	47	99	1.131	1.030	1.242	<0.001
HDL –cho (mg/dL)	51	78	1.078	0.989	1.174	0.118
LDL-cho (mg/dL)	97	134	0.970	0.913	1.031	0.515
UA (mg/dL)	4.0	6.0	0.998	0.917	1.087	0.341
Hb (g/dL)	13.4	15.5	0.663	0.470	0.934	0.050
Ht (%)	39.0	44.6	1.726	1.233	2.417	0.006
WBC (×10³/μl)	4200	5900	0.976	0.909	1.047	0.666
Plt (×10⁴/μl)	19.3	25.7	1.02	0.960	1.084	0.674

BMI: Body mass index, SBP: Systolic Blood Pressure, TP: Total protein, Alb: Albumin, T-Bil: Total bilirubin, AST: Aspartic Aminotransferase, ALT: Alanine Transaminase, LDH: Lactate Dehydrogenase, BUN: Blood Urea Nitrogen, Cre: Creatinine, CRP: C-reactive protein, FBS: Fasting Blood Sugar, HbA1c: Hemoglobin A1c,

TG: Triglyceride, HDL-Cho: Heavy Density Lipoprotein-cholesterol, LDL-Cho: Low Density Lipoprotein-cholesterol, UA: Uric Acid, Hb; Hemoglobin, Ht: Hematocrit, WBC: White Blood Cell, Plt: Platelet. Fold-change are derived from the exponential of the β-coefficient of the model and represent the fold-change in sensitive serum syndecan-1 accompanying a one interquartile increase in each factor.

Figure 1

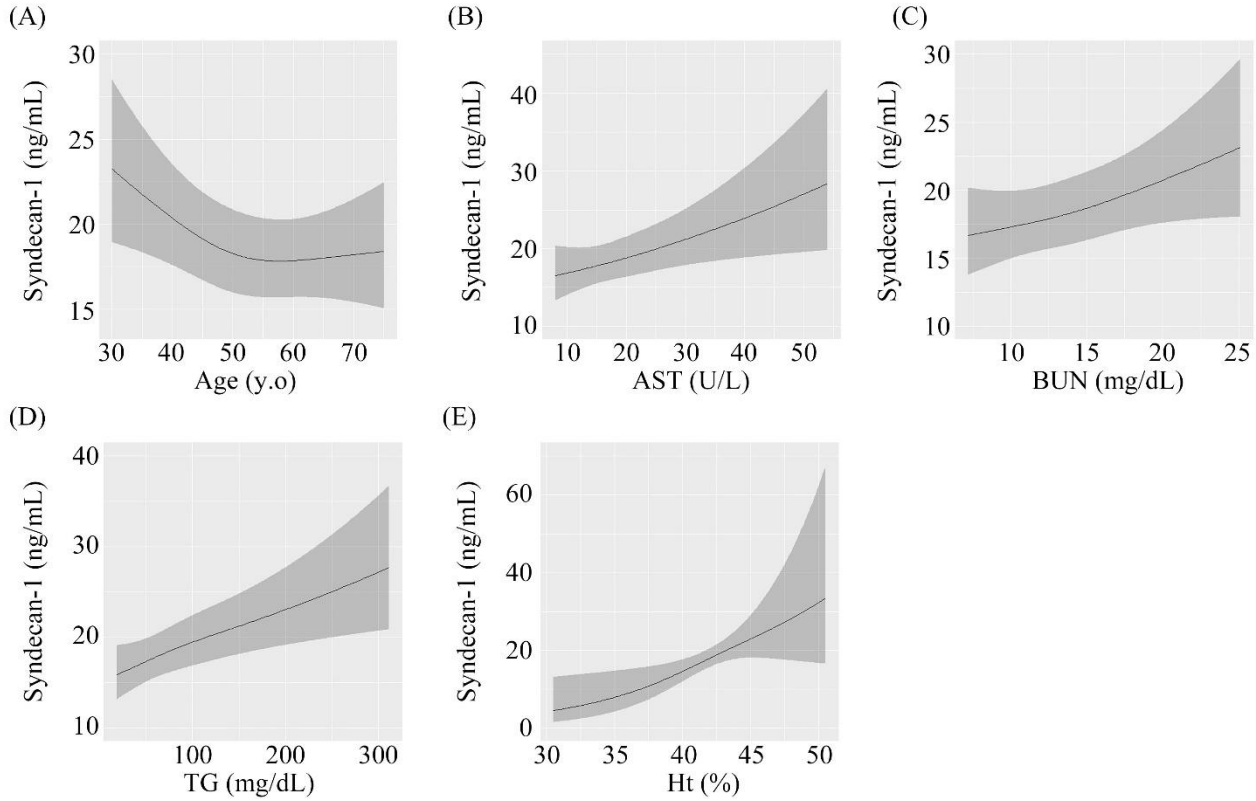

Figure 1: Associations between serum syndecan-1 and different parameters.

Associations of serum syndecan-1 with (A) Age, (B) AST, (C) BUN, (D) triglyceride, and (E) Ht. AST: aspartic aminotransferase, BUN: blood urea nitrogen, Ht: hematocrit

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3.3. Subgroup analysis

Table 3 shows data for the 78 healthy individuals enrolled in this study (that is, individuals receiving no treatment and with no relevant medical history or laboratory data). The median serum syndecan-1 concentration was 19.3 ng/mL (IQR: 13.7–27.3 ng/mL) in healthy participants.

Table 3: Characteristics of the healthy enrollments

BMI: Body mass index, SBP: Systolic Blood Pressure, TP: Total protein, Alb: Albumin, T-Bil: Total bilirubin, AST: Aspartic Aminotransferase, ALT: Alanine Transaminase, LDH: Lactate Dehydrogenase, BUN: Blood Urea Nitrogen, Cre: Creatinine, CRP: C-reactive protein, FBS: Fasting Blood Sugar, HbA1c: Hemoglobin A1c, TG: Triglyceride, HDL-Cho: Heavy Density Lipoprotein-cholesterol, LDL-Cho: Low Density Lipoprotein-cholesterol, UA: Uric Acid, Hb; Hemoglobin, Ht: Hematocrit, WBC: White Blood Cell, Plt: Platelet

	Median or Number	(25-75 Percentile)
Number of Cases	78	
Age	46	(42-52)
Sex (M/F)	41/37	
BMI (kg/m²)	21.9	(20.2-22.8)
SBP (mmHg)	110	(102-119)
TP (g/dL)	7.2	(7.0-7.4)
Alb (g/dL)	4.3	(4.1-4.5)
T-Bil (mg/dL)	0.7	(0.5-0.8)
AST (U/L)	16	(13-19)
ALT (U/L)	14	(10-18)
LDH (U/L)	235	(210-255)
BUN (mg/dL)	12.9	(11.0-14.4)
Cre (mg/dL)	0.72	(0.62-0.86)
CRP (mg/dL)	0.03	(0.01-0.05)
FBS (mg/dL)	94	(88-98)
HbA1c (%)	5.3	(5.2-5.5)
TG (mg/dL)	63	(49-84)
HDL –cho (mg/dL)	67	(57-81)
LDL-cho (mg/dL)	105	(90-117)
UA (mg/dL)	4.7	(4.0-5.6)
Hb (g/dL)	14.2	(13.5-15.0)
Ht (%)	41.1	(39.2-43.7)
WBC (×10³/μl)	5150	(4525-5875)
Plt (×10⁴/μl)	22.2	(19.0-24.2)

4. Discussion

Endothelial disorders are closely related to many diseases via atherosclerosis. The endothelial glycocalyx covers the inner surface of the vascular endothelium and regulates leukocyte adhesion [20]; thus, leukocytes cannot adhere to endothelial cells covered with glycocalyx, and endothelial glycocalyx injury may occur prior to atherosclerotic changes. Syndecan-1 is a component of the glycocalyx, and its degradation indicates endothelial injury [24 27 28]. In this study, to detect the initial endothelial cell injury, we investigated syndecan-1 concentrations in patients who received comprehensive medical examinations. Several previous reports have revealed the relationships of syndecan-1 with severe diseases, such as acute kidney injury, chronic kidney disease, cardiac arrest, cardiovascular disease, and sepsis [29-33]. Although serum syndecan-1 concentrations were reported in several previous studies, the patient populations in these studies were small [29-36]. Additionally, serum syndecan-1 concentrations had not been reported in healthy populations. In this study, 78 healthy individuals with no medication history or abnormal laboratory data were enrolled, and serum syndecan-1 concentrations were determined. However, further studies are required for a more detailed assessment.

The current study revealed that increased serum syndecan-1 concentrations were related to serum triglyceride concentrations. Increased serum triglyceride concentrations may influence

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vascular endothelial injury and subsequently affect atherosclerosis. Notably, triglycerides increase plasma viscosity [37], affecting fluid share stress. Because the glycocalyx serves as a mechanosensor for fluid shear stress [38-40], fluid shear stress on endothelial cells affects the endothelial glycocalyx [41-43], and excess shear stress injuries the endothelial glycocalyx. Thus, increasing serum triglyceride concentrations may damage the endothelial glycocalyx directly. Because the current investigation was performed after fasting, serum triglyceride concentrations were not influenced by the consumption of a meal in our study. Similarly, previous reports revealed that elevated Ht increased wall share stress and affected the degradation of glycocalyx [44 45]. Therefore, our results also showed that Ht was related to syndecan-1 concentrations.

AST and BUN were also closely related to serum syndecan-1 concentrations in the current study. Because syndecan-1 was found to enhance acute kidney injury in a previous report [32], our present findings suggested that the relationship between BUN and syndecan-1 may affect kidney injury. Moreover, increasing BUN is related to dehydration similarly to Ht. AST levels are primarily modulated by liver function. Further studies are needed to elucidate the relationships among AST levels, syndecan-1 concentrations, and liver function.

In this study, we found that age was related to syndecan-1 concentrations. Specifically, with increasing age, syndecan-1 concentrations decreased. This result may be related to the decreased endothelial glycocalyx synthesis of endothelial cells with aging.

Our results also showed that increased syndecan-1 concentrations were not related to decreased HbA1c levels. Previous studies have reported that endothelial glycocalyx perturbation is observed in patients with type 1 and type 2 diabetes mellitus [46-48]. However, in our research, most patients did not have diabetes mellitus, and the experimental settings were different from those of previous studies. Moreover, we evaluated syndecan-1 concentrations as a marker of endothelial glycocalyx injury, whereas previous studies measured glycocalyx volume. Overall, our findings and the results of previous studies suggested that increased syndecan-1 concentrations may reflect the microvessel condition [25 26].

This study had some limitations. First, syndecan-1 is expressed not only in the endothelial glycocalyx but also in other organs. However, we did not evaluate the syndecan-1 expression in different organs in this study. Additionally, the endothelial glycocalyx can be injured in response to inflammatory cytokines and other insults; this mechanism was not evaluated in the current study. Further studies are needed to explore these mechanisms.

Although several markers of the endothelial disorder have been reported, no biomarkers for extreme initial endothelial injury have been identified to date. Therefore, evaluation of endothelial glycocalyx injury may reveal the initial endothelial injury, and syndecan-1 concentrations may be a biomarker reflecting such damage.

5. Conclusions

In conclusion, endothelial glycocalyx injury, which is reflected by serum syndecan-1 concentrations, is related to age, hematocrit, aspartic aminotransferase concentration, blood urea nitrogen concentration, and triglyceride concentration. These results suggest that these factors cause the early endothelial injury indicated by endothelial glycocalyx injury. If treatment intervention against these factors is performed as soon as possible, medical expenses can be reduced to quite an extent.

Author Contributions: K.O. and H.O. wrote the manuscript. T. Kojima., F.D., N.M. and G.T. collected the samples of blood. A.S., H.T., R.K., K. Sumi, Kodai Suzuki, C.T., Keiko Suzuki, S.K., K.K., Y.I., H.Y., R.Z., S.S., T.F., Y. Kawaguchi., T.W., T.K., N.Y., T.D., T.Y., H.U., S.Y. measured syndecan-1 using ELISA. T.I. performed statically analysis. S.O. supervised the animal studies. H.O. and G.T. revised and edited the manuscript. All authors read and approved the final manuscript.

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Conflicts of Interest: The authors declare that they have no competing interests.

References

1. Tabit CE, Chung WB, Hamburg NM, Vita JA. Endothelial dysfunction in diabetes mellitus: molecular mechanisms and clinical implications. Rev Endocr Metab Disord. 2010;11(1):61-74.
2. Vaisman BL, Andrews KL, Khong SM, Wood KC, Moore XL, Fu Y, et al. Selective endothelial overexpression of arginase II induces endothelial dysfunction and hypertension and enhances atherosclerosis in mice. PLoS One. 2012;7(7):e39487.
3. Sharma S, Singh M, Sharma PL. Mechanism of attenuation of diabetes mellitus and hypercholesterolemia induced vascular endothelial dysfunction by protein tyrosine phosphatase inhibition. Vascul Pharmacol. 2011;54(3-6):80-87.
4. Goon PK, Lip GY, Boos CJ, Stonelake PS, Blann AD. Circulating endothelial cells, endothelial progenitor cells, and endothelial microparticles in cancer. Neoplasia. 2006;8(2):79-88.
5. Lefer AM. Endothelial dysfunction as a trigger for ischemia-reperfusion injury. J Thromb Thrombolysis. 1997;4(1):63-65.
6. Maniatis NA, Orfanos SE. The endothelium in acute lung injury/acute respiratory distress syndrome. Curr Opin Crit Care. 2008;14(1):22-30.
7. Annuk M, Zilmer M, Fellstrom B. Endothelium-dependent vasodilation and oxidative stress in chronic renal failure: impact on cardiovascular disease. Kidney Int Suppl. 2003(84):S50-S53.
8. Murdaca G, Colombo BM, Cagnati P, Gulli R, Spano F, Puppo F. Endothelial dysfunction in rheumatic autoimmune diseases. Atherosclerosis. 2012;224(2):309-317.
9. Bijl M. Endothelial activation, endothelial dysfunction and premature atherosclerosis in systemic autoimmune diseases. Neth J Med. 2003;61(9):273-277.
10. Thijssen DH, Black MA, Pyke KE, Padilla J, Atkinson G, Harris RA, et al. Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. Am J Physiol Heart Circ Physiol. 2011;300(1):H2-12.
11. Tremblay JC, Pyke KE. Flow-mediated dilation stimulated by sustained increases in shear stress: a useful tool for assessing endothelial function in humans? Am J Physiol Heart Circ Physiol. 2018;314(3):H508-H20.
12. Becker BF, Chappell D, Jacob M. Endothelial glycocalyx and coronary vascular permeability: the fringe benefit. Basic Res Cardiol. 2010;105(6):687-701.
13. Luft JH. Fine structures of capillary and endocapillary layer as revealed by ruthenium red. Fed Proc. 1966;25(6):1773-1783.
14. Rehm M, Zahler S, Lotsch M, Welsch U, Conzen P, Jacob M, et al. Endothelial glycocalyx as an additional barrier determining extravasation of 6% hydroxyethyl starch or 5% albumin solutions in the coronary vascular bed. Anesthesiology. 2004;100(5):1211-1223.
15. Okada H, Takemura G, Suzuki K, Oda K, Takada C, Hotta Y, et al. Three-dimensional ultrastructure of capillary endothelial glycocalyx under normal and experimental endotoxemic conditions. Crit Care. 2017;21(1):261.
16. Ando Y, Okada H, Takemura G, Suzuki K, Takada C, Tomita H, et al. Brain-specific ultrastructure of capillary endothelial glycocalyx and its possible contribution for blood brain barrier. Sci Rep. 2018;8(1):17523.
17. Inagawa R, Okada H, Takemura G, Suzuki K, Takada C, Yano H et al. Ultrastructural alteration of pulmonary capillary endothelial glycocalyx during endotoxemia. Chest. 2018;154(2):317-325.
18. Lee WL, Slutsky AS. Sepsis and endothelial permeability. N Engl J Med. 2010;363(7):689-691.
19. Woodcock TE, Woodcock TM. Revised Starling equation and the glycocalyx model of transvascular fluid exchange: an improved paradigm for prescribing intravenous fluid therapy. Br J Anaesth. 2012;108(3):384-394.

20. Chelazzi C, Villa G, Mancinelli P, De Gaudio AR, Adembri C. Glycocalyx and sepsis-induced alterations in vascular permeability. Crit Care. 2015;19:26.
21. Paulus P, Jennewein C, Zacharowski K. Biomarkers of endothelial dysfunction: can they help us deciphering systemic inflammation and sepsis? Biomarkers. 2011;16 Suppl 1:S11-21.
22. Salmon AH, Satchell SC. Endothelial glycocalyx dysfunction in disease: albuminuria and increased microvascular permeability. J Pathol. 2012;226(4):562-574.
23. Li L, Ly M, Linhardt RJ. Proteoglycan sequence. Mol Biosyst. 2012;8(6):1613-1625.
24. Rehm M, Bruegger D, Christ F, Conzen P, Thiel M, Jacob M, et al. Shedding of the endothelial glycocalyx in patients undergoing major vascular surgery with global and regional ischemia. Circulation. 2007;116(17):1896-1906.
25. 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.
26. Ostrowski SR, Haase N, Muller RB, Møller MH, Pott FC, Perner A, et al. Association between biomarkers of endothelial injury and hypocoagulability in patients with severe sepsis: a prospective study. Crit Care. 2015;19(1):191.
27. 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.
28. Murphy LS, Wickersham N, McNeil JB, Shaver CM, May AK, Bastarache JA, et al. Endothelial glycocalyx degradation is more severe in patients with non-pulmonary sepsis compared to pulmonary sepsis and associates with risk of ARDS and other organ dysfunction. Ann Intensive Care. 2017;7(1):102.
29. Henrich M, Gruss M, Weigand MA. Sepsis-induced degradation of endothelial glycocalix. ScientificWorldJournal. 2010;10:917-923.
30. Kim YH, Nijst P, Kiefer K, Tang WH. Endothelial glycocalyx as biomarker for cardiovascular diseases: mechanistic and clinical implications. Curr Heart Fail Rep. 2017;14(2):117-126.
31. Liborio AB, Braz MB, Seguro AC, Seguro AC, Martins AMC, Braz MBM, et al. Endothelial glycocalyx damage is associated with leptospirosis acute kidney injury. Am J Trop Med Hyg. 2015;92(3):611-616.
32. 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.
33. Padberg JS, Wiesinger A, di Marco GS, et al. Damage of the endothelial glycocalyx in chronic kidney disease. Atherosclerosis. 2014;234(2):335-343.
34. Grundmann S, Fink K, Rabadzhieva L, Bourgeois N, Schwab T, Moser M, et al. Perturbation of the endothelial glycocalyx in post cardiac arrest syndrome. Resuscitation. 2012;83(6):715-720.
35. Muller RB, Ostrowski SR, Haase N, Wetterslev J, Perner A, Johansson PI. Markers of endothelial damage and coagulation impairment in patients with severe sepsis resuscitated with hydroxyethyl starch 130/0.42 vs Ringer acetate. J Crit Care. 2016;32:16-20.
36. Ostrowski SR, Gaini 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.
37. Sloop GD, Weidman JJ, St Cyr JA. Perspective: interesterified triglycerides, the recent increase in deaths from heart disease, and elevated blood viscosity. Ther Adv Cardiovasc Dis. 2018;12(1):23-28.
38. Florian JA, Kosky JR, Ainslie K, Pang Z, Dull RO, Tarbell JM. Heparan sulfate proteoglycan is a mechanosensor on endothelial cells. Circ Res. 2003;93(10):e136-142.
39. Mochizuki S, Vink H, Hiramatsu O, Kajita T, Shigeto F, Spaan JAE, et al. Role of hyaluronic acid glycosaminoglycans in shear-induced endothelium-derived nitric oxide release. Am J Physiol Heart Circ Physiol. 2003;285(2):H722-726.

40. Thi MM, Tarbell JM, Weinbaum S, Spray DC. The role of the glycocalyx in reorganization of the actin cytoskeleton under fluid shear stress: a "bumper-car" model. Proc Natl Acad Sci U S A. 2004;101(47):16483-16488.

41. Arisaka T, Mitsumata M, Kawasumi M, Tohjima T, Hirose S, Yoshida Y. Effects of shear stress on glycosaminoglycan synthesis in vascular endothelial cells. Ann N Y Acad Sci. 1995;748:543-554.

42. Gouverneur M, Spaan JA, Pannekoek H, Fontijn RD, Vink H. Fluid shear stress stimulates incorporation of hyaluronan into endothelial cell glycocalyx. Am J Physiol Heart Circ Physiol. 2006;290(1):H458-2.

43. Zeng Y, Tarbell JM. The adaptive remodeling of endothelial glycocalyx in response to fluid shear stress. PLoS One. 2014;9(1):e86249.

44. Huo Y, Kassab GS. Effect of compliance and hematocrit on wall shear stress in a model of the entire coronary arterial tree. J Appl Physiol. 2009;107(2):500-505.

45. Constantinescu AA, Vink H, Spaan JA. Elevated capillary tube hematocrit reflects degradation of endothelial cell glycocalyx by oxidized LDL. Am J Physiol Heart Circ Physiol. 2001;280(3):H1051-H1057.

46. Nieuwdorp M, Mooij HL, Kroon J, Atasever B, Spaan JAE, Ince C, et al. Endothelial glycocalyx damage coincides with microalbuminuria in type 1 diabetes. Diabetes. 2006;55(4):1127-1132.

47. Nieuwdorp M, van Haeften TW, Gouverneur MC, Mooij HL, van Lieshout MHP, Levi M, et al. Loss of endothelial glycocalyx during acute hyperglycemia coincides with endothelial dysfunction and coagulation activation in vivo. Diabetes. 2006;55(2):480-486.

48. Broekhuizen LN, Lemkes BA, Mooij HL, Meuwese MC, Verberne H, Holleman F, et al. Effect of sulodexide on endothelial glycocalyx and vascular permeability in patients with type 2 diabetes mellitus. Diabetologia. 2010;53(12):2646-2655.