

1 Article

2 **Factors enhancing serum syndecan-1 concentrations:
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20 **Abstract:** Endothelial disorders are related to various diseases. An initial endothelial injury is
21 characterized by endothelial glycocalyx injury. We aimed to evaluate endothelial glycocalyx injury
22 by measuring serum syndecan-1 concentrations in patients during comprehensive medical
23 examinations. A single-center, prospective, observational study was conducted at Asahi University
24 Hospital. The participants enrolled in this study were 1313 patients who underwent comprehensive
25 medical examinations at Asahi University Hospital from January 2018, to June 2018. One patient
26 undergoing hemodialysis was excluded from the study. At enrollment, blood samples were
27 obtained, and study personnel collected demographic and clinical data. No treatments or exposures
28 were conducted except for standard medical examinations and blood sample collection. Laboratory
29 data were obtained by collection of blood samples at the time of study enrolment. According to
30 nonlinear regression, the concentrations of serum syndecan-1 were significantly related to age ($p =$
31 0.016), aspartic aminotransferase concentration (AST, $p = 0.020$), blood urea nitrogen concentration
32 (BUN, $p = 0.013$), triglyceride concentration ($p < 0.001$), and hematocrit ($p = 0.006$). These
33 relationships were independent associations. Endothelial glycocalyx injury, which is reflected by
34 serum syndecan-1 concentrations, is related to age, hematocrit, AST concentration, BUN
35 concentration, and triglyceride concentration.36 **Keywords:** Endothelial disorders; glycocalyx injury; syndecan-1; nonlinear regression.
3738 **1. Introduction**39 Endothelial disorders are closely related to many diseases, including diabetes mellitus [1],
40 hypertension [2], hypercholesterolemia [3], tumorigenesis [4], ischemia/reperfusion injury [5],
41 respiratory disorder [6], renal dysfunction [7], and autoimmune vasculitis [8]. Previous studies have
42 suggested that treatments used for endothelial disorders could also prevent cardiovascular disease
43 [8 9]. Vascular endothelial disorder exists prior to atherosclerosis, and flow-mediated dilation (FMD)
44 after experimentally imposed increases in shear stress can be used as an index of endothelial function
45 [10 11]. However, FMD is not applicable as a screening approach because it requires the use of
46 echography for diagnosis. Although high-sensitivity C-reactive protein (CRP), lipoprotein-associated

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

51 The endothelium exists on the inner surface of blood vessels as a thin monolayer and therefore
52 is exposed to the circulating blood. Endothelial cells in direct contact with circulating blood are called
53 vascular endothelial cells. Vascular endothelial cells line the entire circulatory system, from the heart
54 to the smallest capillaries. All healthy endothelium is coated by the sugar-protein glycocalyx [12-17],
55 which plays key roles in vascular homeostasis, including regulation of microvascular tone and
56 endothelial permeability, maintenance of an oncotic gradient across the endothelial barrier,
57 regulation of the adhesion and migration of leukocytes, and inhibition of intravascular thrombosis
58 [18-20]. The glycocalyx is composed of cell-bound proteoglycans, glycosaminoglycan side chains, and
59 sialoproteins [21-23]. Proteoglycans consist of a core protein, such as a syndecan family protein, to
60 which glycosaminoglycan is linked. Syndecan-1 is the core protein in heparan sulfate proteoglycan,
61 which is also found in the glycocalyx. Syndecan-1 is released from the endothelium upon injury to
62 the glycocalyx, causing its concentration in the circulation to increase [24]. In fact, serum syndecan-1
63 was used as an endothelial injury marker in recent clinical studies of sepsis [25 26].

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

66 2. Experimental Section

67 2.1. Study population

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

70 2.2 Ethics approval and consent to participate

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

74 2.2 Consent for publication

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

76 2.3 Clinical assessments

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

81 2.4. Laboratory data

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

85 2.5. Statistical analyses

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

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

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

105

106 **3. Results**

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

110 *3.1. Characteristics of the patients*

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

117 **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/m²)	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 (× 10³/μl)	5000	(4200-5900)
Plt (× 10⁴/μl)	22.4	(19.3-25.7)
History of Present Illness		
Hypertension	234	(17.8%)
Diabetes Mellitus	80	(6.1%)
Hyperlipidemia	173	(13.2%)
Hyper Uric Acid	70	(5.3%)
Malignant Neoplasm	65	(5.0%)

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

123

124 *3.2. Associations of serum syndecan-1 with various parameters*

125 The results of multivariable regression analysis are shown in Table 2. Age, AST concentration,
 126 BUN concentration, triglyceride concentration, and Ht were significantly associated with serum
 127 syndecan-1 concentrations after adjustment for sex, medication and BMI. A 1 IQR increase in age was
 128 independently associated with a 0.9-fold increase in serum syndecan-1 ($\beta = 0.903$; 95% confidence
 129 interval [CI]: 0.831–0.982; $p = 0.016$; Fig 1A). Similar results were found for AST ($\beta = 1.093$; 95% CI:
 130 0.996–1.200; $p = 0.020$; Fig 1B), BUN ($\beta = 1.083$; 95% CI: 1.018–1.152; $p = 0.013$; Fig 1C), triglyceride (β
 131 = 1.131; 95% CI: 1.030–1.242; $p < 0.001$; Fig 1D) and Ht ($\beta = 1.726$; 95% CI: 1.233–2.417; $p = 0.006$; Fig
 132 1E) after adjustment for covariates. No significant associations were observed for other factors.

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

137

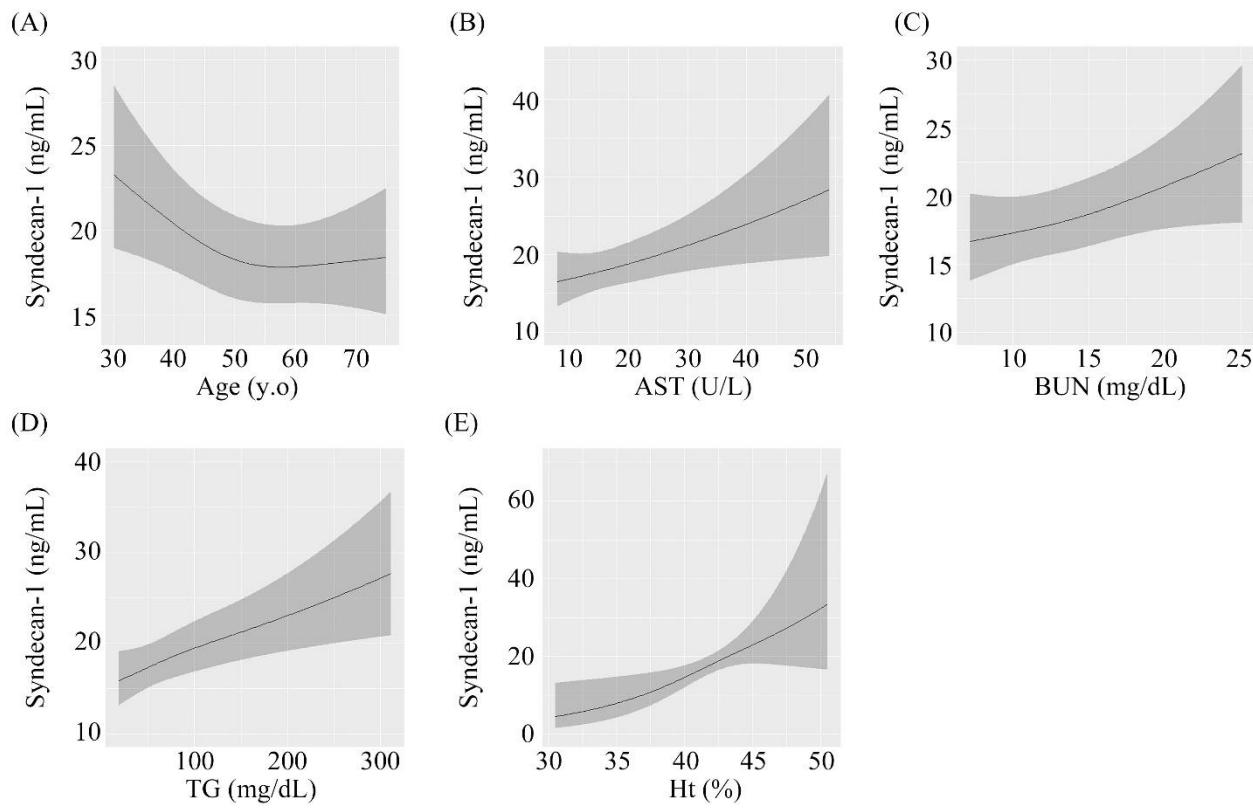
138 **Table 2: Results of multivariable regression analysis**

	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 ($\times 10^3/\mu\text{L}$)	4200	5900	0.976	0.909	1.047	0.666
Plt ($\times 10^4/\mu\text{L}$)	19.3	25.7	1.02	0.960	1.084	0.674

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

142 TG: Triglyceride, HDL-Cho: Heavy Density Lipoprotein-cholesterol, LDL-Cho: Low Density Lipoprotein-
143 cholesterol, UA: Uric Acid, Hb: Hemoglobin, Ht: Hematocrit, WBC: White Blood Cell, Plt: Platelet. Fold-
144 change are derived from the exponential of the β -coefficient of the model and represent the fold-change in
145 sensitive serum syndecan-1 accompanying a one interquartile increase in each factor.
146

Figure 1



147
148 **Figure 1: Associations between serum syndecan-1 and different parameters.**
149 Associations of serum syndecan-1 with (A) Age, (B) AST, (C) BUN, (D) triglyceride, and (E) Ht. AST:
150 aspartic aminotransferase, BUN: blood urea nitrogen, Ht: hematocrit
151

152 *3.3. Subgroup analysis*

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

156 **Table 3: Characteristics of the healthy enrollments**

157 BMI: Body mass index, SBP: Systolic Blood Pressure, TP: Total protein, Alb: Albumin, T-Bil: Total bilirubin, AST:
 158 Aspartic Aminotransferase, ALT: Alanine Transaminase, LDH: Lactate Dehydrogenase, BUN: Blood Urea

	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)

159 Nitrogen, Cre: Creatinine, CRP: C-reactive protein, FBS: Fasting Blood Sugar, HbA1c: Hemoglobin A1c, TG:
 160 Triglyceride, HDL-Cho: Heavy Density Lipoprotein-cholesterol, LDL-Cho: Low Density Lipoprotein-
 161 cholesterol, UA: Uric Acid, Hb: Hemoglobin, Ht: Hematocrit, WBC: White Blood Cell, Plt: Platelet

162 **4. Discussion**

163 Endothelial disorders are closely related to many diseases via atherosclerosis. The endothelial
 164 glycocalyx covers the inner surface of the vascular endothelium and regulates leukocyte adhesion
 165 [20]; thus, leukocytes cannot adhere to endothelial cells covered with glycocalyx, and endothelial
 166 glycocalyx injury may occur prior to atherosclerotic changes. Syndecan-1 is a component of the
 167 glycocalyx, and its degradation indicates endothelial injury [24 27 28]. In this study, to detect the
 168 initial endothelial cell injury, we investigated syndecan-1 concentrations in patients who received
 169 comprehensive medical examinations. Several previous reports have revealed the relationships of
 170 syndecan-1 with severe diseases, such as acute kidney injury, chronic kidney disease, cardiac arrest,
 171 cardiovascular disease, and sepsis [29-33]. Although serum syndecan-1 concentrations were reported
 172 in several previous studies, the patient populations in these studies were small [29-36]. Additionally,
 173 serum syndecan-1 concentrations had not been reported in healthy populations. In this study, 78
 174 healthy individuals with no medication history or abnormal laboratory data were enrolled, and
 175 serum syndecan-1 concentrations were determined. However, further studies are required for a more
 176 detailed assessment.

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

179 vascular endothelial injury and subsequently affect atherosclerosis. Notably, triglycerides increase
180 plasma viscosity [37], affecting fluid shear stress. Because the glycocalyx serves as a mechanosensor
181 for fluid shear stress [38-40], fluid shear stress on endothelial cells affects the endothelial glycocalyx
182 [41-43], and excess shear stress injures the endothelial glycocalyx. Thus, increasing serum
183 triglyceride concentrations may damage the endothelial glycocalyx directly. Because the current
184 investigation was performed after fasting, serum triglyceride concentrations were not influenced by
185 the consumption of a meal in our study. Similarly, previous reports revealed that elevated Ht
186 increased wall shear stress and affected the degradation of glycocalyx [44 45]. Therefore, our results
187 also showed that Ht was related to syndecan-1 concentrations.

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

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

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

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

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

214 5. Conclusions

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

221 **Author Contributions:** K.O. and H.O. wrote the manuscript. T. Kojima., F.D., N.M. and G.T. collected the
222 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.,
223 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
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230 **Conflicts of Interest:** The authors declare that they have no competing interests.

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