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Syndecan-1 Levels and Early Positive Fluid Balance Are Associated With Disease Severity in Acute Pancreatitis

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Objective: The aim of the study is to study fluid balance and endothelial glycocalyx degradation, reflected by syndecan-1, and heparan sulfate (HS) levels, in early stages of acute pancreatitis (AP).

Materials and Methods: This study comprised of 210 AP patients (104 mild, 53 moderately severe, 17 severe). Blood was sampled within 72 hours from the onset of symptoms, and plasma syndecan-1 and HS levels were determined using ELISA. Fluid balance up to sampling and up to 4 days was determined retrospectively from medical records.

Results: Syndecan-1 levels predicted severe AP (SAP) in receiver operating characteristic analysis [area under curve 0.699, 95% confidence interval (CI) 0.546 to 0.851, $P = 0.021$]. Increasing AP severity was associated with higher intravenous fluid intake and lower urine output. In multivariate binary logistic regression analysis, positive fluid balance up to sampling [odds ratio (OR) 1.05 per 100 ml, 95% CI 1.02 to 1.11, $P = 0.010$] and higher Acute Physiology and Chronic Health Evaluation II score at sampling (OR 1.48, 95% CI 1.20 to 1.83, $P < 0.001$) were independently associated with severe AP, while syndecan-1 level was not.

Conclusions: SAP is associated with high positive fluid balance in the early stages of treatment. Although increased in SAP, syndecan-1 was not independently associated with SAP when controlling for fluid balance and Acute Physiology and Chronic Health Evaluation II score.

Key Words: acute pancreatitis, fluid resuscitation, fluid balance, endothelium, glycocalyx

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Acute pancreatitis (AP) is an inflammatory condition of the pancreas, most frequently resulting from excessive alcohol use or bile stones. Most AP cases are mild (MAP), but 10–20% of the patients develop severe AP (SAP), which is often complicated by organ dysfunction (OD) caused by systemic inflammation or infected pancreatic necrosis, and associated with mortality ranging from 17% up to 52%.[1–3] Among the main challenges in treating AP successfully are the lack of both definitive treatment as well as reliable biomarkers predicting the severity of AP. Intravenous (IV) fluid resuscitation remains the cornerstone of supportive treatment in AP as well as in sepsis. International guidelines recommend Ringer acetate to be used in fluid resuscitation. Recommendations regarding the volume or rate of administration of IV fluids are however based on weak evidence[4] with some evidence supporting the use of aggressive fluid therapy in MAP and a more restricted approach in SAP.[5,6] A recent meta-analysis found no difference in mortality in AP patients between aggressive and restricted fluid resuscitation, but aggressive fluid therapy was associated with increased risk of renal and respiratory complications.[7] Vascular endothelium may represent a source of biomarker candidates in systemic inflammatory diseases like AP and sepsis, as disruption of the vascular endothelial glycocalyx has been described in these diseases.[8] Aggressive intravenous fluid therapy can result in further disintegrity of the glycocalyx, and regarding that the glycocalyx disruption directly promotes vascular permeability, interstitial edema with severe consequences may follow.[5,9]

The glycocalyx is a gel-like layer covering the luminal surface of vascular endothelial cells. It is composed of membrane-bound proteoglycans, glycoproteins, glycosaminoglycans, and adherent plasma proteins. It maintains homeostasis of the vasculature by controlling vascular permeability and microvascular tone, preventing microvascular thrombosis, and regulating leukocyte adhesion to endothelium.[9] In sepsis, biomarker potential of shed syndecan-1, one of the glycocalyx proteoglycans, and the glycosaminoglycan heparan sulfate (HS), has recently been studied quite intensively. For example, elevated plasma levels of syndecan-1 have been shown to be associated with 90-day mortality.[10] There is also evidence of association between syndecan-1 levels and excessive fluid therapy, kidney failure, and the risk of intubation.[11] In sepsis and septic shock, plasma HS has been reported to predict in-hospital mortality and to correlate with the amount of administered intravenous fluids[12] and both HS and syndecan-1 have been shown to associate with disease severity and occurrence of disseminated intravascular coagulation due to progressive endothelial damage.[8]

One study suggests that rapid rate of fluid administration is associated with increased sepsis and mortality among SAP patients.[13] Despite shared features such as the systemic inflammation, such as the release of cytokines tumor necrosis factor alpha and interleukin 6,[14] and the treatment with high fluid volumes in both sepsis and AP, syndecan-1 and HS have not been studied in AP. The aim of the current study was to examine the relationships between AP severity, syndecan-1 and HS plasma levels, and fluid balance. We hypothesized that these markers are elevated and correlate with disease severity and the administered volume of fluid during the early stages of AP.

MATERIALS AND METHODS

Patients

A total of 255 AP patients were recruited at Helsinki University Hospital emergency unit from December 2013 to May 2019.

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Patients aged 18 years or older were included in the study if their symptoms had started ≤48 hours before admission. Patients with chronic pancreatitis, known malignancy, or several (≥3) previous AP attacks were excluded from the study.

The primary study questions of this study were the association of syndecan-1 and HS with severity of AP and the fluid balance at sampling and at day four.

The secondary study questions included the association of fluid balance at sampling and on day four with severity of AP.

AP was diagnosed as described in the revised Atlanta classification: symptoms typical to AP and plasma amylase levels more than three times the normal upper limit and/or findings typical to AP in computed tomography (CT).[4] The final severity of AP was retrospectively classified from patient documents according to the revised Atlanta classification. MAP patients had no local or systemic complications. Moderately severe AP (MSAP) patients had local complications diagnosed with CT or magnetic resonance imaging (MRI), transient OD lasting less than 48 hours, or their underlying chronic illness was aggravated. SAP patients had persistent OD lasting over 48 hours.[15]The presence of OD (pulmonary, renal, or cardiovascular) was assessed using the Modified Marshall Score (MMS).[16]

Written informed consent was acquired from all included patients or their next of kin. The study protocol (HUS/1685/2016) was approved by the Surgical Ethical Review Board (Joint Authority of the Helsinki and Uusimaa Hospital District) as an amendment to an earlier protocol approving patient recruitment and acquisition of blood samples for study of inflammatory mechanisms in AP.

Fluid Therapy

Fluid therapy received by the patients was assessed retrospectively from electronic patient records (Uranus, PICIS). Type of fluid was recorded. Total volume of fluids was calculated for each 24-hours period ending at 24:00. Of note, the fluid volume of the admission day represents the total volume from admission to 24:00. Subsequent daily fluid intake was recorded from 00:00 to 24:00. The fluid balance at sampling was calculated by subtracting the urine volume, other measurable losses (excretion via nasogastric tube in the intensive care unit [ICU]) and 1000 ml/d for evaporation from the amount of fluid received intravenously and orally, and it was reported per hour as the time to sampling was variable. Reliable fluid administration data up to sampling was available for 200 patients (137 MAP, 51 MSAP, and 12 SAP). The total urine output and amount of IV fluids received were also recorded for up to 4 days. As all patients did not receive IV fluids for the full 4 days, the amounts were reported per day. Reliable fluid data up to 4 days was available for 206 patients (139 MAP, 53 MSAP, and 14 SAP).

Plasma Biomarkers

A venous blood sample was obtained from all patients within 72 hours from the onset of symptoms. Plasma was separated and stored at −80°C until the measurements. Plasma levels of syndecan-1 and HS were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits [Human Syndecan-1 ELISA Kit (CD138) ab46506 (Abcam) and Human HS (Heparan Sulfate) ELISA Kit E-EL-H2364 (Elabscience), respectively] according to the manufacturers' instructions. Plasma sample was available for 210 patients (140 MAP, 53 MSAP, and 17 SAP).

Statistics

Continuous data are shown as means with SD or median with interquartile ranges, and categorical data as numbers and percentages. The variables analyzed in the Results section were found not to be normally distributed by Kolmogorov-Smirnov test (data not shown). AP severity groups are compared using Jonckheere-Terpstra test with post hoc Mann-Whitney tests. Correlations were calculated using Spearman correlation test. Predictive value of variables for development of SAP was assessed using receiver operating characteristic (ROC) analysis and the independent association of the variables with outcome was assessed using multivariate binary logistic regression. We considered $P < 0.05$ as statistically significant. We used SPSS version 27 (IBM, Chicago, IL) for statistical software for all analyses.

RESULTS

Altogether, 210 patients were included in the study. The flow chart demonstrating the number of patients in analyses is presented in Figure 1. Characteristics and clinical features of all patient groups are shown in Table 1. Local complications, as defined in Atlanta classification, were peripancreatic fluid collections at sampling. Other local complications, that is, walled of necrosis and pancreatic pseudocysts, developed later. Infectious complications also developed after sampling. Results of routine clinical laboratory tests taken at admission and at the time of sampling of plasma for syndecan-1 and HS are shown in Table 2. Blood hematocrit, leukocytes, and hemoglobin were higher in SAP patients at admission and at sampling after fluid therapy had already commenced.

Plasma Biomarkers

The mean time of sampling for the study variables was 16 hours (SD 8 h, range 1–48 h) from hospital admission. Syndecan-1 levels were higher in the SAP group than in the MAP and MSAP groups (Fig. 2A). No significant differences were found in HS levels (Fig. 2A). In the total patient population, plasma syndecan-1 level detected SAP (n = 12) with ROC area under the curve (AUC) of 0.699 (95% CI 0.546 to 0.851, $P = 0.021$: Fig. 2B).

Among patients with no OD at sampling (n = 183), plasma syndecan-1 level did not predict SAP (n = 7) [ROC AUC 0.586 (95% CI 0.386 to 0.786, $P = 0.441$)], nor did plasma HS level [ROC AUC 0.698 (95% CI 0.466 to 0.930, $P = 0.076$)].

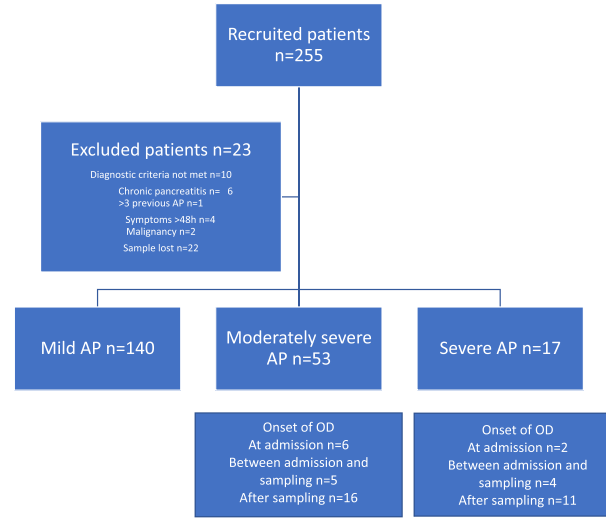

FIGURE 1. Flow chart of patient recruitment.

TABLE 1. Characteristics of the Patients

	Severity of Acute Pancreatitis		
	Mild (n = 140; 67%)	**Moderately Severe (n = 53; 25%)**	**Severe (n = 17; 8%)**
Male sex, n (%)	94 (67%)	35 (66%)	14 (82%)
Age (y)	51.6 (16.5)	54.0 (14.2)	50.5 (15.6)
Etiology of AP, n (%)			
Alcohol	74 (53%)	31 (59%)	12 (71%)
Biliary	34 (24%)	12 (23%)	3 (18%)
Post-ERCP	2 (1%)	3 (6%)	0 (0%)
Idiopathic	21 (15%)	6 (11%)	1 (6%)
Other	9 (6%)	1 (2%)	1 (6%)
Hospital length of stay (d)	4.4 (2.2)	10.2 (5.5)	34.7 (27.7)
ICU admission, n (%)	0 (0%)	5 (9%)	16 (94%)
ICU length of stay (d)	NA	3.8 (1.5)	19.1 (16.2)
Organ dysfunction			
Respiratory	0	27 (51%)	17 (100%)
Renal	0	26 (49%)	11 (65%)
Cardiovascular	0	1 (2%)	6 (35%)
30-day mortality, n (%)	0 (0%)	0 (0%)	8 (47%)
SOFA score on admission	0.9 (1.1)	1.7 (1.7)	3.8 (3.0)
SOFA score at sampling	1.1 (1.2)	2.2 (1.6)	5.0 (2.8)
APACHE II score on admission	5.2 (2.9)	6.6 (3.3)	8.1 (3.9)
APACHE II score at sampling	4.9 (3.3)	7.0 (3.1)	9.2 (4.2)
APACHE II without age points on admission	3.0 (2.0)	4.2 (2.7)	5.9 (4.2)
APACHE II without age points at sampling	2.7 (2.3)	4.6 (2.6)	7.0 (4.3)
MMS on admission	0.2 (0.4)	0.5 (0.7)	0.8 (1.1)
MMS at sampling	0.4 (0.6)	0.8 (0.8)	1.7 (1.4)
Time from onset to admission (h)	20.0 (13.5)	16.8 (15.3)	20.9 (16.5)
Time from onset to sampling (h)	36.4 (15.0)	32.8 (16.4)	35.3 (18.1)
First AP, n (%)	107 (77%)	38 (72%)	16 (94%)
Local complication, n (%)	0 (0%)	29 (55%)	13 (77%)
Pancreatic necrosis, n (%)	0 (0%)	20 (38%)	15 (88%)
CT, n (%)	76 (55%)	44 (83%)	16 (92%)
Underlying conditions			
Cardiac insufficiency	0	1 (2%)	1 (6%)
Renal insufficiency	2 (2%)	2 (4%)	0
Hypertension	9 (7%)	1 (2%)	1 (6%)
Atherosclerosis	9 (7%)	1 (2%)	1 (6%)
Coronary heart disease	7 (5%)	1 (2%)	1 (6%)
COPD	2 (1%)	3 (6%)	0
Asthma	18 (13%)	6 (11%)	0
Diabetes	21 (15%)	3 (6%)	3 (18%)

Data shown as mean (standard deviation) or number of patients (percentage).

COPD indicates chronic obstructive pulmonary disease; ERCP, endoscopic retrograde cholangio-pancreatography; NA, not available; SOFA, sequential organ failure assessment.

Intravenous Fluid Therapy and Urine Output

IV fluid therapy consisted of crystalloids (Ringer acetate, glucose 5% or 0.9% saline) during the first 24 hours. Increasing disease severity was associated with higher intravenous fluid (Fig. 3A) and lower urine output (Fig. 3B) up to sampling. Consequently, the fluid balance was significantly associated with AP severity (Fig. 3C).

Patients who developed severe AP received more fluids per day during the 4-day follow-up (Fig. 4A), and their urine output was lower (Fig. 4B). In some mild cases, IV fluids were discontinued before day 4 because of rapid recovery.

Syndecan-1 Levels in Conjunction to Fluid Therapy and Urine Output

Syndecan-1 levels correlated weakly with the total amount of IV fluid received per day up to 4 days (Table 3) and HS levels correlated weakly with fluid balance up to sampling (Table 3).

TABLE 2. Laboratory Parameters (Blood/Plasma) on Hospital Admission and at Sampling

Time	Laboratory Test	Acute Pancreatitis Severity			*P*
		Mild (n = 140)	Moderately Severe (n = 53)	Severe (n = 17)	
Admission	**Hemoglobin, g/l**	144 (133–154)	148 (135–161)	158 (149–168)	0.004
	Hematocrit, %	42 (39–44)	43 (39–45)	45 (43–48)	0.005
	Leukocyte count, $\times 10^9$/l	11.0 (8.7–13.8)	12.8 (9.2–16.1)	16.3 (12.5–20.1)	<0.001
	Platelet count, $\times 10^9$/l	239 (200–302)	216 (156–278)	204 (153–250)	0.002
	Sodium, mmol/l	138 (134–140)	138 (135–140)	137 (132–139)	0.668
	Potassium, mmol/l	3.8 (3.5–4.0)	3.7 (3.5–4.0)	3.8 (3.6–3.9)	0.870
	C-reactive protein, mg/l	11 (3–41)	5 (3–18)	15 (3–40)	0.392
	Creatinine, µmol/l	69 (60–81)	67 (56–83)	86 (69–145)	0.136
Sampling	**Hemoglobin, g/l**	133 (123–142)	135 (126–160)	154 (149–162)	<0.001
	Hematocrit, %	39 (36–42)	40 (37–45)	44 (42–47)	0.001
	Leukocyte count, $\times 10^9$/l	10 (7.8–12.5)	10.8 (8.5–15.8)	14.3 (12.5–15.9)	0.001
	Platelet count, $\times 10^9$/l	216 (176–272)	188 (137–240)	164 (139–222)	<0.001
	Sodium, mmol/l	138 (135–140)	137 (135–139)	135 (132–138)	0.049
	Potassium, mmol/l	3.7 (3.4–3.8)	3.7 (3.5–4.1)	4 (3.8–4.3)	<0.001
	C-reactive protein, mg/l	53 (17–116)	64 (20–153)	187 (52–209)	0.009
	Creatinine, µmol/l	63 (53–73)	62 (50–73)	103 (69–163)	0.192
	Syndecan-1, ng/ml	39 (26–64)	49 (30–111)	64 (42–171)	0.003
	Heparan sulfate, ng/ml	0.53 (0.35–1.18)	0.61 (0.41–0.97)	0.85 (0.31–3.48)	0.275

Medians with interquartile ranges. *P* value from Jonckheere-Terpstra test.

IV Fluid Therapy in Persistent OD

Since both plasma syndecan-1 level and positive fluid balance up to sampling were associated with the development of SAP, they were entered in a logistic regression model. Acute Physiology and Chronic Health Evaluation II (APACHE II) without age score was added to reflect severity of acute disease in the model with age as a separate variable. Among patients with no OD at sampling and among all patients, volume of IV fluid and APACHE II without age score predicted SAP (Table 4). Syndecan-1 level was not an independent predictor of SAP.

DISCUSSION

IV fluid resuscitation is the mainstay in the management of acute pancreatitis (AP), even though it may induce damage to the vascular endothelium by disrupting its glycocalyx layer and impairing vascular permeability. In this study, we determined plasma levels of the glycocalyx components syndecan-1 and HS in patients with AP and showed that syndecan-1, but not HS, is associated with disease severity. However, the predictive ability of syndecan-1 was poor, and it was not independently associated with SAP when controlling for other measures of disease severity. Patients who received high volumes of IV fluids also had a higher risk for persistent OD. Furthermore, low urine output was associated with disease severity already in the early stages of the disease. To our knowledge, this is the first study to investigate the relations between syndecan-1, HS, IV fluid administration, and disease severity in patients with AP.

Previous studies on sepsis have shown that syndecan-1 levels correlate with disease severity,[11] and our study shows that this is the case also in AP. However, our finding that HS does not correlate with disease severity in AP is not in accordance with previous results concerning sepsis.[8,12] HS is degraded from syndecan-1 molecules by heparinase, which is activated by tumor necrosis factor and angiopoietin-2.[17] This mechanism is thought to induce endothelial dysfunction and contribute to OD in sepsis. Heparanase, along with inflammatory mediators, activate metalloproteinases that contribute to the shedding of syndecan-1.[18] Among these is matrix metalloproteinase (MMP) 9, which has been shown to be elevated in severe AP.[19] Syndecan-1 shedding is thus induced by various inflammatory pathways, which might explain why syndecan-1 but not HS correlated with disease severity close to the onset of symptoms in AP.

In addition, in contrast to studies on sepsis,[11,12,20,21] syndecan-1 and HS levels did not correlate with fluid balance in our study. This might be explained by the more extensive microvascular and macrovascular damage and stronger immunological response in rapidly aggravating sepsis compared to early stages of AP.[14,22,23] It can be hypothesized that syndecan-1 and HS levels would rise and correlate better to fluid balance later in SAP due to necrotizing component of AP, but that remains to be shown. While there is evidence that large volume of resuscitative fluid can contribute to hypervolemia and concomitant shedding of the glycocalyx,[24,25] in our study, syndecan-1 levels did not correlate with fluid balance. This could be interpreted that syndecan-1 levels in SAP are higher because of changes induced by SAP or that SAP patients are more vulnerable to glycocalyx injury caused by aggressive fluid therapy.

We also found that those AP patients who had high APACHE II score and received higher volumes of IV fluids were in greater risk to develop persistent OD. A recent retrospective study showed that increased fluid balance is associated with increased risk of OD and mortality in AP patients treated in ICU[26] and, while mild AP patients have been shown to benefit from early aggressive fluid resuscitation,[6] our study supports the findings that too aggressive fluid therapy could be harmful to patients in risk of developing SAP.[13] Thus, the AP patients that respond poorly to initial fluid resuscitation could benefit from more intensive observation and support rather than just more aggressive IV fluid administration. There is indeed accumulating evidence that sepsis patients with OD could benefit from a more restricted fluid strategy.[27]

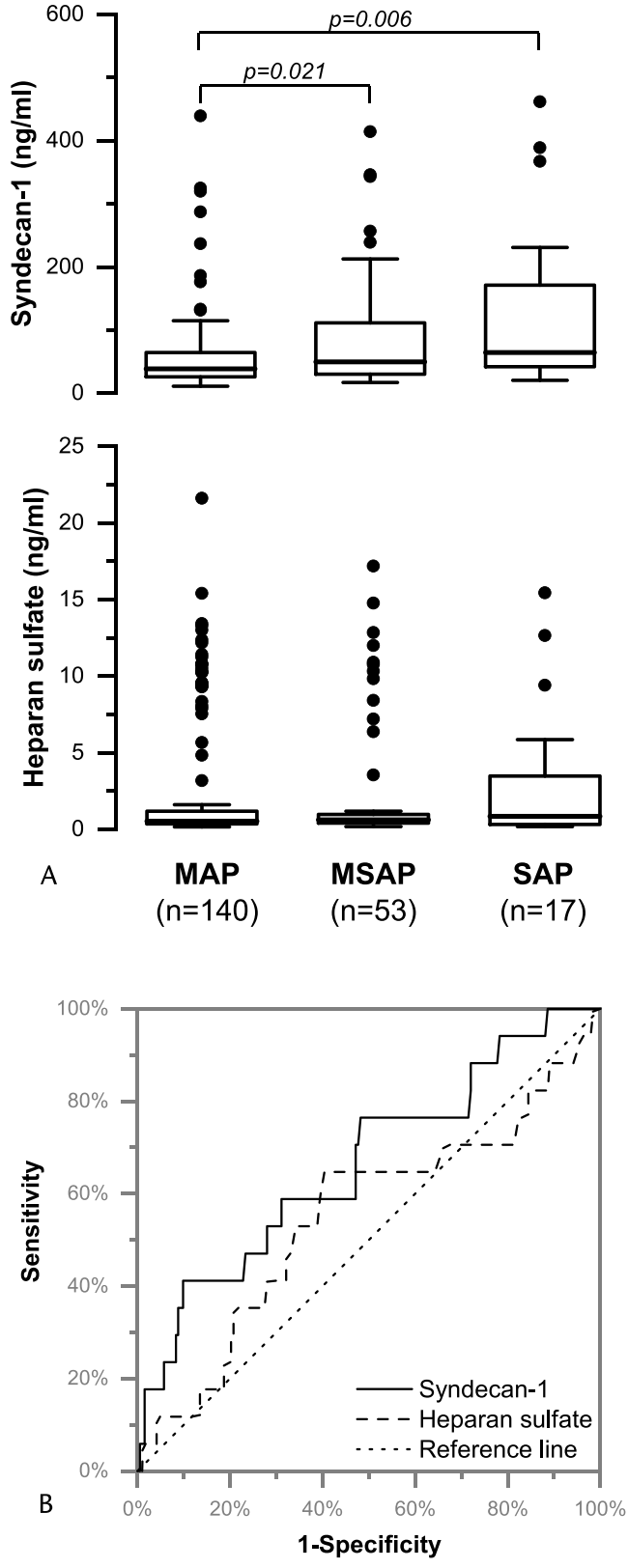

FIGURE 2. A, Plasma levels of syndecan-1 and heparan sulfate in patients with MAP), MSAP, and SAP. B, ROC curves for prediction of SAP in all patients. Groups compared using Jonckheere-Terpstra test, statistically significant *post hoc* Mann-Whitney test *P* values shown. Boxes denote 25th to 75th percentiles (interquartile range [IQR]) with medians shown as horizontal lines. Outliers (over 1.5 times IQR) are shown as dots, with one extreme outlier in syndecan-1 MAP group not shown but included in the analysis.

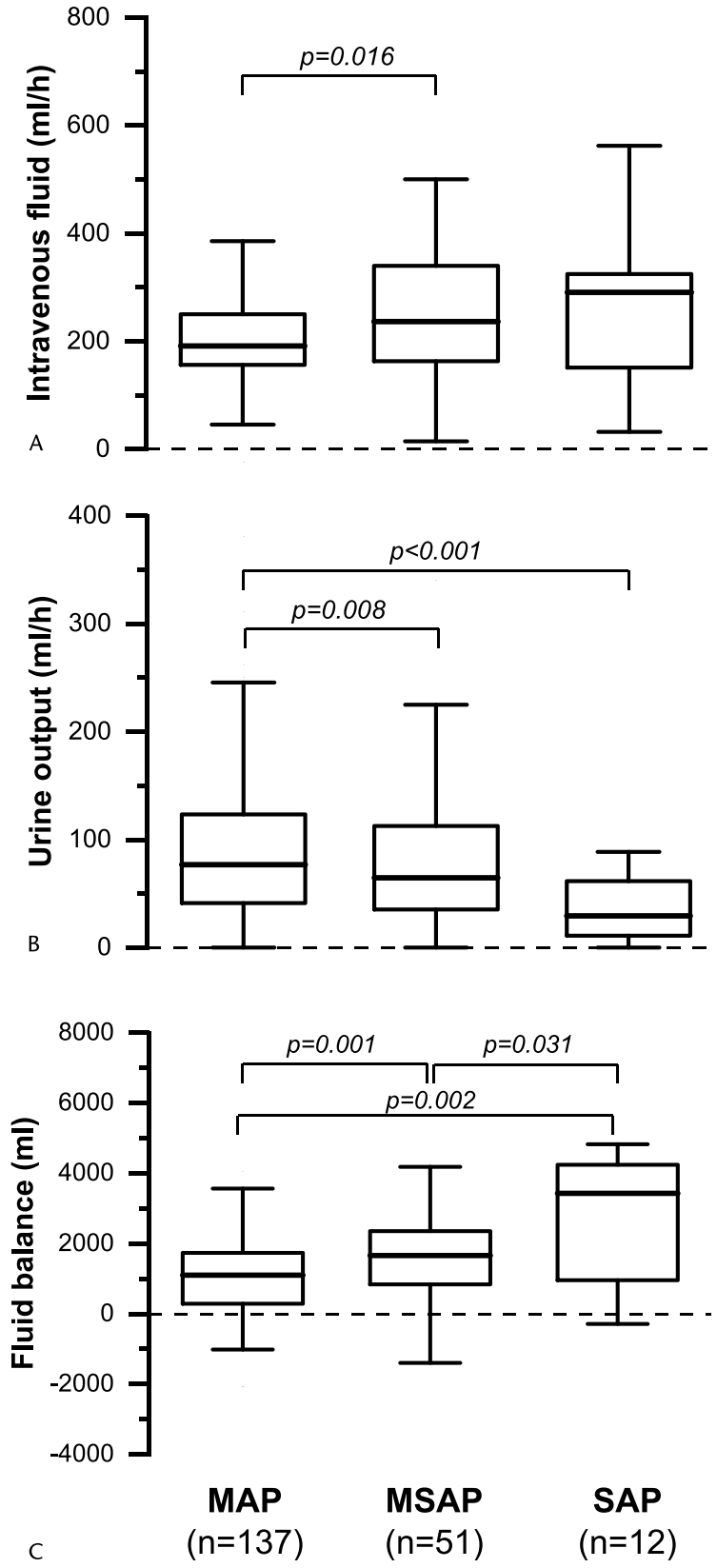

FIGURE 3. A, Intravenous fluid intake. B, Urine output. C, Fluid balance up to sampling in patients with MAP, MSAP, and SAP acute pancreatitis. Groups compared using Jonckheere-Terpstra test, statistically significant *post hoc* Mann-Whitney test *P* values shown. Boxes denote 25th to 75th percentiles with medians shown as horizontal lines and extreme values as whiskers.

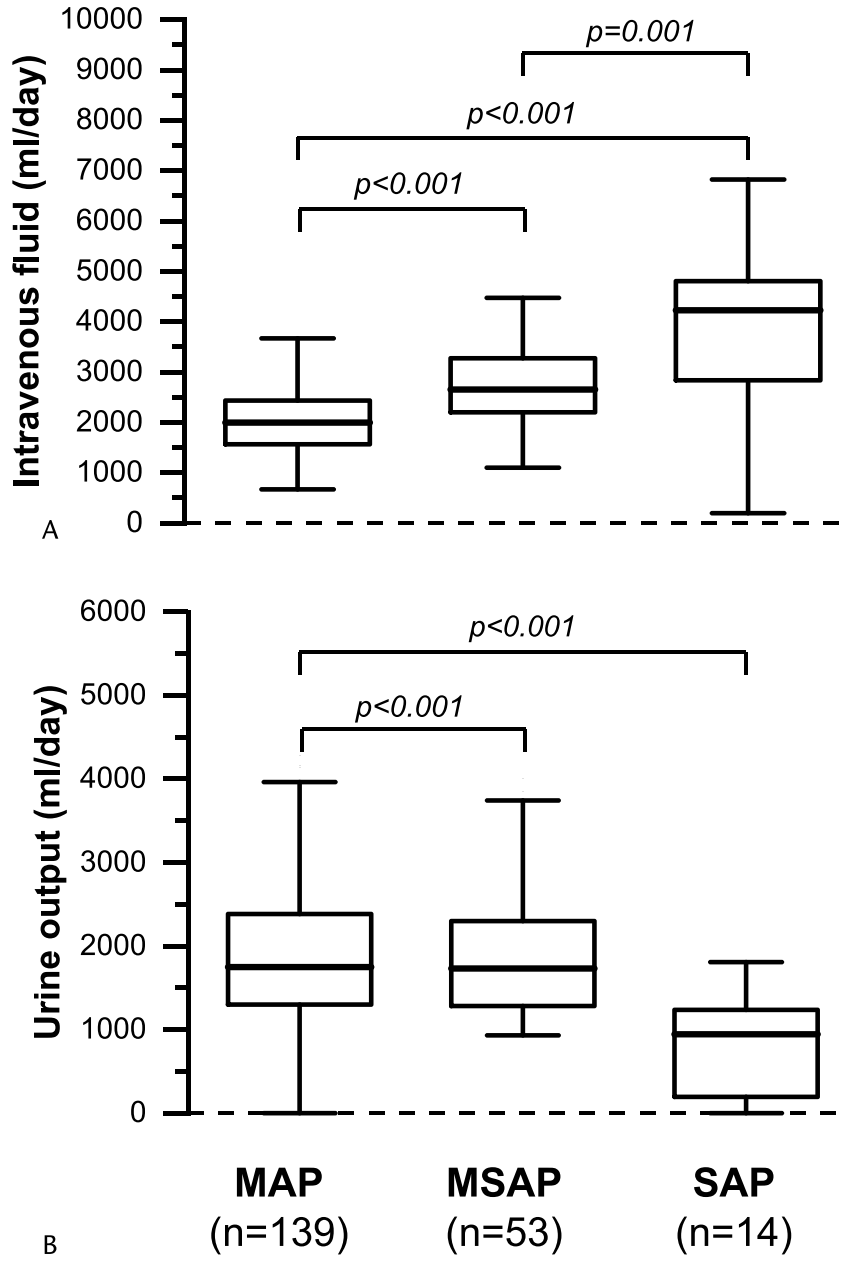

FIGURE 4. A, Intravenous fluid intake and B, urine output up to 4 days in patients with MAP, MSAP, and SAP acute pancreatitis. Groups compared using Jonckheere-Terpstra test, statistically significant *post hoc* Mann-Whitney test *P* values shown.

TABLE 3. Correlation of Syndecan-1 and Heparan Sulfate With IV Fluids and Urine Output

	Syndecan-1	Heparan Sulfate
Total iv fluids up to sampling, ml/h	0.072	0.112
Total urine output up to sampling, ml/h	−0.124	−0.068
Fluid balance up to sampling, ml	0.087	0.151 ($P = 0.034$)
Total iv fluids days 1–4, ml/d	0.153 ($P = 0.028$)	−0.028
Total urine output days 1–4, ml/d	−0.124	−0.080

Spearman correlation coefficients with *P* values where statistically significant.

TABLE 4. Multivariate Binary Logistic Regression for Severe Acute Pancreatitis With Variables at Sampling

	Variables in Model	OR (95% CI)	*P*
No OD at sampling	Fluid balance (per +100 ml)	1.07 (1.02 to 1.12)	**0.010**
(total 186, persistent OD 7)	Plasma syndecan-1 (per 100 ng/ml)	1.11 (0.76 to 1.61)	0.603
	APACHE II without age points	1.45 (1.16 to 1.81)	**0.001**
	Age (y)	1.00 (0.94 to 1.05)	0.846
All patients	Fluid balance (per +100 ml)	1.05 (1.01 to 1.10)	**0.026**
(total 200, persistent OD 12)	Plasma syndecan-1 (per 100 ng/ml)	1.23 (0.85 to 1.78)	0.269
	APACHE II without age points	1.48 (1.20 to 1.83)	**<0.001**
	Age (y)	0.98 (0.94 to 1.03)	0.508

It is also notable that whereas the AP patients that developed persistent OD received more fluids and their urine secretion was lower, their hematocrit and hemoglobin values were also higher after initial fluid resuscitation as compared to the other patients. This suggests that fluid leakage outside the vascular system occurs already in the early stages of SAP, which by definition is AP with persistent OD.

We have previously searched for predictive biomarkers for SAP in the early phases of the disease from leukocyte signaling molecules[28,29] and MMPs[30] with promising results. Instead, according to the present results, syndecan-1 seems to have only limited use and HS none in predicting AP severity in the early stages of the disease. Hence, syndecan-1 and HS are not likely to be potential predictive biomarkers to be adapted for clinical use. We agree with a previously presented conclusion that better biomarkers of glycocalyx injury (than syndecan-1) need to be further searched.[20] However, the results of this study suggest that endothelial glycocalyx is degraded during the early disease, and we cannot exclude that aggressive fluid therapy may play a role.

This retrospective laboratory analysis clinical study has its limitations. First, the analysis of syndecan-1 and HS was secondary, and the study was not initially planned to assess their association with severity of AP or fluid volume. Second, because of the relative rarity of SAP, only 17 patients developed SAP in our study and therefore the number of SAP patients in all analyses was small, and we cannot fully exclude type 2 error in our results. Third, we concentrated on the early phases of the disease so that possible associations of syndecan-1 and HS levels to fluid balance later in SAP and disease outcome warrant more studies. Importantly, because disease severity and endothelial permeability are interdependent, the volume of fluid needed in resuscitation phase of severe cases is a function of both, which may affect the strength of associations in statistical models. It is also worth noting that because development of infectious complications is not uncommon during the course of MSAP and SAP, it can obscure the interpretation concerning associations between plasma biomarker levels and "pure" AP.

We conclude that a high positive fluid balance in early AP is associated with the development of persistent OD. Syndecan-1 levels also show an association with disease severity in AP; however, when adjusted for disease severity scores, this association is not found to be independent. Our results emphasize the need for studies where fluid balance data are collected prospectively, as the retrospective approach in the present study was associated with severe limitations.

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