Individualized Early Goal-Directed Therapy in Systemic Inflammation: Is Full Utilization of Preload Reserve the Optimal Strategy?

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Objectives: In severe acute pancreatitis, the administration of fluids in the presence of positive fluid responsiveness is associated with better outcome when compared to guiding therapy on central venous pressure. We compared the effects of such consequent maximization of stroke volume index with a regime using individual values of stroke volume index assessed prior to severe acute pancreatitis induction as therapeutic hemodynamic goals.

Design: Prospective, randomized animal study.

Setting: University animal research laboratory.

Subjects: Thirty domestic pigs.

Interventions: After randomization, fluid resuscitation was started 2 hours after severe acute pancreatitis induction and continued for 6 hours according to the respective treatment algorithms. In the control group, fluid therapy was directed by maximizing stroke volume index, and in the study group, stroke volume index assessed prior to severe acute pancreatitis served as primary hemodynamic goal.

Measurements and Main Results: Within the first 6 hours of severe acute pancreatitis, the study group received a total of 1,935.8±540.7 mL of fluids compared with 3,462.8±828.2 mL in the control group ($p < 0.001$). Pancreatic tissue oxygenation did not differ significantly between both groups. Vascular endothelial function, measured by flow-mediated vasodilation before and 6 hours after severe acute pancreatitis induction, revealed less impairment in the study group after treatment interval (−90.76% [study group] vs −130.89% [control group]; $p = 0.046$). Further, lower levels of heparan sulfate (3.41±5.6 pg/mL [study group] vs 43.67±46.61 pg/mL [control group]; $p = 0.032$) and interleukin 6 (32.18±8.81 pg/mL [study group] vs 77.76±56.86 pg/mL [control group]; $p = 0.021$) were found in the study group compared with control group. Histopathological examination of the pancreatic head and corpus at day 7 revealed less edema for the study group compared with the con-

trol group (1.82±0.87 [study group] vs 2.89±0.33 [control group, pancreatic head]; $p = 0.03$; 2.2±0.92 [study group] vs 2.91±0.3 [control group, pancreatic corpus]; $p = 0.025$).

Conclusions: Individualized optimization of intravascular fluid status during the early course of severe acute pancreatitis, compared with a treatment strategy of maximizing stroke volume by fluid loading, leads to less vascular endothelial damage, pancreatic edema, and inflammatory response. (*Crit Care Med* 2014; 42:e741–e751)

Key Words: endothelial dysfunction; fluid management; glycocalyx damage; inflammation; pancreatitis; volume responsiveness

Severe acute pancreatitis (SAP) is an enzyme-mediated self-digestion of the pancreas and is associated with high mortality (1, 2). About 15–20% of patients with an acute pancreatitis progress to a hemorrhagic-necrotizing form with organ destruction and systemic inflammatory response syndrome associated with prolonged disease course (3). Characteristic pathophysiologic changes are hyperdynamic circulatory failure, vasodilatation, endothelial dysfunction, capillary leakage, and intravascular volume depletion (4).

Recently, studies dealt with different fluid management strategies in SAP (5–7). Optimization of intravascular fluid status is a cornerstone in the early therapy of diseases associated with systemic inflammation (8). The introduction of novel concepts of advanced hemodynamic monitoring and the development of differentiated treatment algorithms for improving macrocirculation have intensified the debate regarding the most favorable fluid management (9, 10). Several studies revealed that improved microcirculation results in less histopathologic organ destruction and improved survival (7, 11–14). However, for maintenance of adequate microcirculatory perfusion, maintaining macrocirculation is indispensable. In particular, in clinical situations associated with systemic inflammation, optimization of cardiac preload has been shown to be essential to ensure adequate cardiac output and sufficient tissue oxygenation (15, 16). Several studies demonstrated that volume therapy guided to maximize cardiac output, that is, to consequently use preload reserve by repeated volume loading until no further increase in stroke volume (SV) and cardiac output can be achieved, resulted in improved outcome in critically ill patients (9, 16, 17). However, there is increasing experimental and clinical evidence that volume overload also leads to impaired outcome in particular in critical illness with systemic inflammation (7, 18, 19).

Complete utilization of preload reserve is also rather an unphysiological state. Hence, we hypothesized that in the early state of SAP even more beneficial to guide fluid therapy oriented on individualized, "physiological" goals of SV rather than to utilize the complete preload reserve. This strategy may lead to less damage of the endothelial glycocalyx and in consequence to less deleterious effects on the pancreatic tissue. Therefore, we compared in this study two different treatment algorithms for individualized early goal-directed fluid therapy in a porcine experimental model of SAP during the 6 hours after SAP induction. The algorithm of control group (CG) was based on optimizing and maximizing stroke volume index (SVI). The study group (SG) algorithm aimed at maintaining SVI at individual values measured at baseline, that is, prior to induction of SAP.

Endpoints were the tissue oxygenation of the pancreas (tpO_2), the vascular endothelial dysfunction, the glycocalyx degradation, the range of systemic inflammation, and the pancreatic tissue damage.

MATERIALS AND METHODS

Study Design

This prospective randomized trial in 30 domestic pigs was approved by the Governmental Commission on the Care and Use of Animals of the city of Hamburg. The animals received care in compliance with the "Guide for the Care and Use of Laboratory Animals." The study was carried out according to the Animal Research Reporting In Vivo Experiments Guidelines (20).

Anesthesia and Surgical Preparation

Animals arrived 7 days before the experimental protocol started and received an antibiotic prophylaxis (tulathromycin, benzathin-benzylpenicillin, dihydrostreptomycinsulfat) over 3 days according to the standards of the facility. Each animal was fasted overnight and received ketamine 15 mg/kg, midazolam 0.5 mg/kg, azaperone 2 mg/kg, and atropine 0.5 mg. Animals received an IV catheter in the ear vein and were connected to an electrocardiogram and pulse oximetry. After orotracheal intubation, anesthesia was maintained by continuous infusion of fentanyl (50 µg/kg/hr) and inhalation of sevoflurane (end-expiratory concentration, 2.0%). A volume-controlled ventilation (10 mL/kg) and a positive end-expiratory pressure of 5 cm H_2O (Zeus; Drägermedical, Lübeck, Germany) were performed. Ventilator frequency was adjusted to maintain end-expiratory pCO_2 of 35–40 mm Hg. Continuous infusion rate of 6 mL/kg of balanced solution (Sterofundin, B. Braun, Melsungen, Germany) was maintained in both groups. Body temperature was kept constant using warming blankets.

A 7.5F and a 12F venous catheter were inserted in the external jugular vein. A 5F Thermistor-tipped arterial catheter (Pulsiocath, Pulsion Medical Systems, Feldkirchen, Germany) was placed in the femoral artery and connected to a dedicated hemodynamic monitor (PiCCO 2, Pulsion Medical Systems). An abdominal laparotomy was performed. Pancreas and duodenum were mobilized, whereupon meticulous attention was paid to strainless positioning and preservation of organ perfusion. To quantify tpO_2, a polarographic measuring probe (Licox CC1.P1 combined oxygen and temperature probe, Integra, Hempshire, Great Britain) was inserted in the pancreatic tissue at intersection of pancreatic head and corpus.

Hemodynamic Management

Two different treatment strategies were applied. In the SG, volume therapy and hemodynamic stabilization were guided by SVI and heart rate (HR) as assessed at baseline before induction of SAP. Therefore, HR and SVI were recorded at baseline and defined as an animal's optimal SVI and HR (SVI_{opt} and HR_{opt}).

In case of a decrease in SVI of greater than 15% below SVI_{opt}, or alternatively an increase in HR of greater than or equal to 50%, a volume loading step (VLS) (hydroxyethyl starch 6% and Ringer's solution at a fixed ratio on 1:1, 7 mL/kg) was applied (**Fig. 1**). In the CG, volume therapy and hemodynamic stabilization were guided by maximizing SVI. After induction of SAP, SVI was optimized by repeated VLS. VLS was repeated until SVI did not increase anymore by more than 15% (**Fig. 2**).

In both groups, continuous infusion of norepinephrine was initiated when mean arterial pressure decreased below 60 mm Hg.

Measurements and Experimental Protocol

All hemodynamic variables were measured by transcardiopulmonary thermodilution (PiCCO 2, Pulsion Medical Systems). After baseline measurements (M0), induction of SAP was accomplished by infusion of 1.6 mL/kg glycodeoxycholic acid (10 mmol/L, pH 8, Sigma, Steinheim, Germany) into the pancreatic duct via an IV catheter (Vasofix 0.8 mm, B. Braun) using an automated infusion system (PerfusorFM [MFC], B. Braun) during a period of 10 minutes to ensure standardized infusion pressure and simultaneous application of cerulein (2 µg/kg) IV (17). This model mimics the development of an ascending SAP caused by duct obstruction, beginning in the pancreatic head and spreading all over the entire organ when the inflammation disperses. The pancreatic duct was ligated and measurements were performed 60 minutes after SAP induction (M1). Measurements were repeated after another 60 minutes (M2), and fluid management according to the different treatment protocols was initiated. Thereafter, measurements were repeated 30 minutes after initiation of the different treatment strategies (M3), and then repeated every 60 minutes during the further course of the experimental protocol (M4–M8) (**Fig. 3**). At each time point, arterial and central venous blood gas analyses were defined. At M0, M2, and M8, additional venous blood samples were taken for analysis of heparan sulfate, syndecan-1, and blood counts. After completion of intraoperative measurements (M8), all catheters were removed except for the central venous catheter, which was cannulated to the dorsal neck of the animals for further application of analgesic medication and sampling of blood in the postoperative course. After wound closure and extubation, the animals were brought to a prewarmed barn in the animal facility.

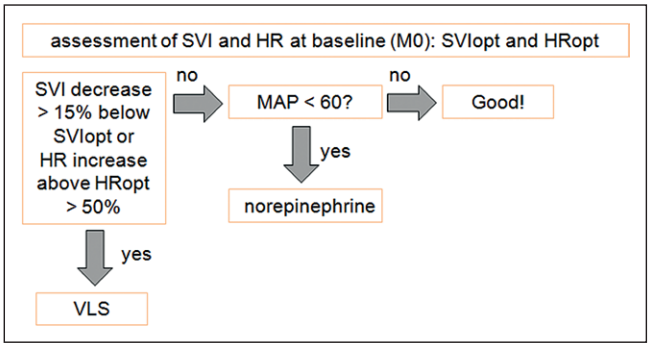

Figure 1. Treatment algorithm based on individualized goals for stroke volume index (SVI) in the study group. HR = heart rate, MAP = mean arterial pressure, VLS = volume loading step.

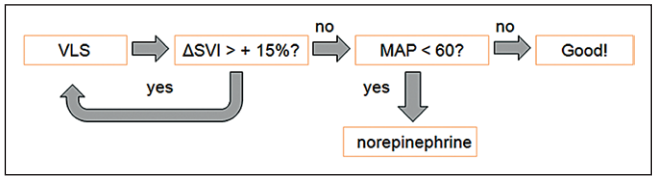

Figure 2. Treatment algorithm based on maximizing stroke volume index (SVI) in the control group. MAP = mean arterial pressure, VLS = volume loading step.

Flow-Mediated Vasodilation

Flow-mediated vasodilation (FMD) is a noninvasive ultrasonic measurement to quantify vasomotor function of peripheral artery diameters. FMD serves as a surrogate for endothelial nitric oxide bioavailability (21). FMD was assessed in the femoral artery in supine position with fixed leg at M0 and M8 with a high-frequency linear array transducer (10 MHz, GE, Vivid i, Wauwatosa, WI). The femoral artery was prepared without manipulating the vessel itself. Images were received simultaneously with electrocardiographic tracing and were digitally recorded (three cardiac cycles). At the beginning of each measurement, the femoral artery was identified and baseline images were taken. The artery diameter from posterior to anterior was measured during diastole at the best angle of interrogation to determine the intima-media thickness. A vessel clip was positioned proximally on the artery to interrupt the blood flow for 5 minutes. Sixty and 120 seconds after clip removal and reperfusion, the vessel diameter was measured to assess the vasodilator response. The mean of nine measurements of baseline and posthyperemia diameters was used for statistical analysis. FMD was expressed as the relative change in the artery diameter during hyperemia and defined as 100 × ([posthyperemia diameter – baseline diameter]/baseline diameter) (21). Quantification of FMD was performed offline by an investigator blinded to the group membership of the respective animal.

Glycocalyx Degradation and Inflammatory Response

Serum concentrations of heparan sulfate and syndecan-1, the two predominant endothelial glycosaminoglycans, were quantified by enzyme-linked immunosorbent assay (ELISA) (heparan sulfate: amsbio, Abingdon, United Kingdom; syndecan-1: Diaclone SAS, Besançon Cedex, France) (22). An elevation of serum values of these glycocalyx components indicates a diminution of the endothelial glycocalyx accompanied by increased capillary permeability and enhanced tissue edema (23). Blood samples taken on M0 and M8 to measure heparan sulfate and syndecan-1, and further blood samples taken on M9 to quantify interleukin (IL)-6, were centrifuged and the serum was stored at –80°C. The concentration of IL-6 was measured by using the Porcine IL-6 Quantikine ELISA Kit (R&D Systems, Minneapolis, MN) and a microtiter reader (Tecan, Männedorf, Schweiz) at 450 nm. IL-6 was measured on postoperative day 1 to detect the peak of IL-6 (24, 25).

Postoperative Observation

From the first postoperative day, animals had free access to food and water. Analgesia was ensured by administration of

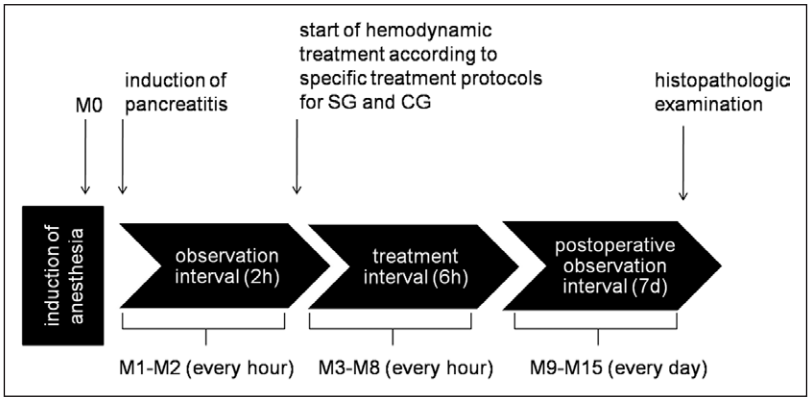

Figure 3. Overview of the study protocol. After induction of anesthesia, M0 was performed (baseline measurement of hemodynamic variables, flow-mediated vasodilation [FMD], heparan sulfate). After that, severe acute pancreatitis induction started with an observation interval over 2 hr (M1 and M2). Accordingly, the different treatment protocols were initiated and obtained for 6 hr (treatment interval, M3–M8). At M8, second FMD and second measurement of heparan sulfate were conducted. M9–M15 is the postoperative observation interval with interleukin-6 gauging at M9. At M15, the histopathologic examinations were performed. CG = control group, SG = study group.

piritramid (15 mg) every 6 hours via the central venous catheter. A ward round was performed four times a day, once a day by a veterinarian of the animal facility. Blood gas analyses and blood samples were taken once a day. Furthermore, a porcine well-being and fitness score were surveyed (26). On the postoperative day 7, animals were reanesthetized and killed by injection of T61 (200 mg embutramid, 50 mg mebezonium, 5 mg tetracain/mL) during deep anesthesia. Sampling of tissue specimens from pancreatic head, corpus, and tail was performed. In animals, which died during the observation period, autopsy and tissue sampling were conducted immediately after death. The histological specimens were stored in 3.5% formalin.

Histopathologic Examination

Specimens were examined by a treatment-blinded experienced pathologist. The histopathologic evaluation of the pancreatic lesions based on a previous publication was slightly modified to improve standardization (11) (**Table 1**). Histopathologic changes were evaluated for each pancreatic area, that is, head, corpus, and tail, separately, and for each anatomic region a total score ranging from 0 (no alterations) to 12 (severe pancreatitis) was determined. Counts of inflammatory cells were performed at high-power field (HPF) measuring 0.3068 mm^2. To determine the score of intralobular inflammation, 10 randomly selected HPFs were viewed and an average value of cells per HPF was calculated. Enzymatic necrosis of pancreatic fat tissue was defined as pink shadow cells with finely granular cytoplasm, lacking a nucleus. For total parenchymal necrosis, a score of 3 was assigned for the classification of edema and inflammation, respectively.

Statistical Analysis

Data were analyzed using SPSS for Windows (IBM, SPSS Statistics version 20.0, Armonk, NY). All data were tested for normal distribution using the Kolmogorov-Smirnov test. Parametric variables are expressed as mean and SD. For comparison of differences between the groups, a one-way analysis of variance (ANOVA)/*t* test in combination with a Levene test were used or, in case of not normally distributed data, the nonparametric Mann-Whitney *U* test was used. For analysis of differences between the groups in repeated measurements, the variance analysis for repeated measurements (ANOVA) followed by post hoc test (least significant difference) were used. Significance was appraised for a p value of less than 0.05.

RESULTS

Study Population

In both groups, 15 animals were studied. One animal in the CG was excluded because of intraoperative death. Animals did not present any significant difference regarding age, body length, or weight between the treatment groups ($p > 0.05$). Mean body length and body weight were 97.3 ± 4.8 cm, 29.3 ± 3.6 kg (SG) and 97.0 ± 3.5 cm, 31.5 ± 2.8 kg (CG).

TABLE 1. Histopathologic Scoring System for Porcine Pancreatitis

Score	Acinar Necrosis	Fatty Tissue Necrosis	Intralobular Inflammation (Plasma Cells, Lymphocytes, and Granulocytes Outside Parenchymal and Fatty Tissue Necrosis)	Edema
0	Nil	Nil	Nil	Nil
1	< 10 necrotic acinar cells/lobule	< 1/3 of plane	Loose infiltrates (≤ 30 cells/HPF)	Interlobular edema
2	≥ 10 necrotic acinar cells/lobule	≥ 1/3 to < 2/3 of plane	Moderate infiltrates (> 30; ≤ 100 cells/HPF)	Interacinar edema, ≥ 2 lobules
3	≥ 1/3 of plane	≥ 2/3 of plane	Dense infiltrates (> 100 cells/HPF)	Intercellular edema, ≥ 2 lobules

HPF = high-power field.

Histopathologic pancreatitis total score ranges from 0 (no alterations) to maximal 12 points (severe pancreatitis); an HPF measures 0.3068 mm^2.

Hemodynamic Management and Fluid Therapy

The course of hemodynamic variables, fluid therapy, and norepinephrine administration are presented in **Table 2**. In total, the animals of the SG received 30 VLSs. Twelve of those 30 VLSs were applied, according to the treatment algorithm, because of an increase in HR by more than 50%.

Tissue Oxygenation of the Pancreas

The course of tissue oxygenation and blood gas analysis is presented in **Table 3**, showing no significant differences between both groups during the treatment interval.

From M3 to M8, hemoglobin was significantly higher in the SG compared with the CG. Lactate and pH did not show significant differences during the treatment interval.

Endothelial Function

FMD. In both groups, FMD was studied in 10 animals. In the other five animals per group, no optimal conditions for measurement could be achieved. FMD at M0 did not differ between both groups (6.49% ± 5.08% [SG] 8.58% ± 8.45% [CG]; $p > 0.05$). When compared with M0, FMD significantly decreased in both groups at M8 (0.6% ± 2.3%, $p = 0.022$ [SG] vs –2.65% ± 2.97%, $p = 0.019$ [CG]). For the intergroup comparison, significant results between the groups at M8 were found. In the SG, the FMD decreases about –90.76% and in the CG about –130.89% ($p = 0.046$) (**Fig. 4**).

Glycocalyx Degradation and Inflammatory Response. Plasma concentration of heparan sulfate at M0 revealed no difference between both groups. As illustrated in **Figure 5**, values of heparan sulfate were significantly lower at M8 in the SG compared with the CG (3.41 ± 5.6 pg/mL for SG vs 43.67 ± 46.61 pg/mL for CG; $p = 0.032$). For syndecan-1, no statistically significant differences between the groups were shown (18.05 ± 5.62 pg/mL [CG] vs 29.98 ± 6.79 pg/mL [SG] at M0; $p > 0.05$; 11.73 ± 6.90 pg/mL vs 13.62 ± 10.05 pg/mL at M8; $p > 0.05$).

There was no significant difference in the leukocyte count at M0, M2, and M8–M15. At M0, the CG values of IL-6 were below the detection limit (0 ± 0) (minimum detectable dose, 2.03 pg/mL) and thereby significantly lower IL-6 values in the SG (10.41 ± 3.04 pg/mL; $p < 0.05$). At M9, IL-6 was significantly lower in the SG compared with the CG (32.18 ± 8.81 pg/mL for SG vs 77.76 ± 56.86 pg/mL for CG; $p = 0.021$) (**Fig. 6**).

Histopathologic Examination

Tissue samples showed differences in histopathologic findings (**Table 4**).

Postoperative Observation

Evaluation of the porcine well-being score, the fitness score, and the survival revealed no difference between both groups. In both groups, two animals died during the observation time of 7 days.

DISCUSSION

The focus of this study was to compare the effects of volume therapy by merely maintaining individual normal values of SVI versus consequent maximization of SVI based on the concept of volume responsiveness in an experimental model of systemic inflammation. Our model closely mimics the clinical course of SAP and enables evaluation of the therapeutic effect on macrocirculation and tissue oxygenation. Maximizing SV by repeated volume loading apparently aggravated endothelial dysfunction and enhanced endothelial glycocalyx degradation and inflammatory response and led to significantly more pancreatic edema compared to a treatment strategy aiming toward maintaining individual, "normal" values of SVI in SAP.

The concept of maximizing SVI by repeated volume loading has been advocated to allow individual optimization of macrohemodynamics, in particular in the peri- and postoperative care of high-risk surgical patients. However, probatory fluid loading not necessarily leads to an increase in SV; it also frequently leads to a negative fluid response, that is, SVI does not increase following this volume load (27, 28). In order to avoid this unnecessary and potentially harmful volume loading in the presence of fluid responsiveness, functional variables such as pulse pressure variation and stroke volume variation were advocated since they allow predicting fluid responsiveness (7). Consequently, guiding therapy by those parameters leads to an, in fact nonphysiological, complete use of preload reserve. In contrast to this approach, our consideration was to guide therapy orientated on an individual goal of SVI, which reflects a state of "physiological fluid responsiveness." In this hypothesis-generating study, we chose the individual SVI value, which was assessed prior to SAP induction, during hemodynamic stability, which of course is in this form not directly transferable in clinical reality, but allows investigating our hypothesis of individualized targets.

With regard to oxygen supply and tissue oxygenation reflecting microcirculatory blood flow, no differences were present between the SG and the CG. Also no differences for serum lactate and $ScvO_2$ were found. These results suggest comparable microcirculatory conditions in both groups. As recently described by De Backer et al (29, 30), adequate macrocirculation is an essential prerequisite, but microcirculation is obviously to a large extent independent from macrocirculation. There is considerable evidence that microcirculatory alterations can occur or persist even after the optimization of macrocirculatory variables (31–33). Chappell et al (34) demonstrated the need to preserve the glycocalyx to improve microcirculatory oxygen distribution in critical illness. This stresses the importance of microcirculation and the aim to put all emphasis on prevention of endothelial dysfunction.

To further characterize endothelial dysfunction and endothelial damage, we measured the FMD and the serum concentrations of heparan sulfate and syndycan-1. In humans, FMD technique has been extensively investigated in several cardiovascular scenarios as valid method for detection of endothelial dysfunction (35–37). Application of ultrasound-based techniques for endothelial evaluation in patients with sepsis has not been fully explored. Few studies describe FMD alterations in patients with sepsis (21, 38). In both groups, a significant deterioration of FMD occurred during the treatment interval. But the SG showed a significantly better FMD while SAP

TABLE 2. Hemodynamic Data and Fluid Balance

Variable	Group	M0	M1	M2
Crystalloid fluid (mL)	CG	231 ± 99	425 ± 66	566 ± 65
	SG	252 ± 77	437 ± 73	596 ± 83
Colloid fluid (mL)	CG	0	0	0
	SG	0	0	0
Urine volume (mL)	CG	161 ± 146	311 ± 175	356 ± 163
	SG	122 ± 107	329 ± 212	419 ± 240
Norepinephrine (μg/kg/min)	CG	0	0	0
	SG	0	0	0
Heart rate (min^{-1})	CG	76 ± 9	80 ± 11	84 ± 24
	SG	75 ± 8	79 ± 11	82 ± 11
Mean arterial pressure (mm Hg)	CG	54 ± 5	55 ± 5	55 ± 9
	SG	57 ± 09	55 ± 5	58 ± 7
Stroke volume variation (%)	CG	8.0 ± 2.3	8.1 ± 3.1	9.0 ± 5.1
	SG	9.5 ± 2.7	9.5 ± 2.8	9.8 ± 2.4
Pulse pressure variation (%)	CG	9.7 ± 2.3	9.8 ± 3.2	10.7 ± 4.0
	SG	11.3 ± 2.6	11.4 ± 2.9	11.7 ± 2.7
Central venous pressure (mm Hg)	CG	3.4 ± 2.7	2.6 ± 1.6	3.0 ± 2.0
	SG	3.1 ± 2.0	2.8 ± 1.7	2.9 ± 1.7
Stroke volume index (mL/m^2)	CG	44.2 ± 4.6	41.6 ± 5.1	41.4 ± 8.4
	SG	46.0 ± 6.5	43.1 ± 7.9	44.0 ± 9.3
Cardiac index (L/min)	CG	3.4 ± 0.4	3.3 ± 0.2	3.4 ± 0.3
	SG	3.3 ± 0.5	3.2 ± 0.4	3.2 ± 0.4
Global end-diastolic volume index (mL/m^2)	CG	623 ± 75	593 ± 78	588 ± 101
	SG	601 ± 77	571 ± 75	560 ± 80
Extravascular lung water index (mL/kg)	CG	19.2 ± 1.8	19.7 ± 1.7	19.7 ± 1.8
	SG	19.7 ± 3.5	20.6 ± 3.7	20.1 ± 3.6
Systemic vascular resistance (dynes·s/cm^5/m^2)	CG	1,260 ± 188	1,277 ± 173	1,280 ± 220
	SG	1,280 ± 241	1,226 ± 248	1,306 ± 277
Lactate (mmoL/L)	CG	2.02 ± 0.81	1.51 ± 0.54	1.29 ± 0.37
	SG	1.65 ± 0.75	1.44 ± 0.41	1.2 ± 0.26
Hemoglobin (g/dL)	CG	9.0 ± 0.6	9.0 ± 0.7	8.9 ± 0.7
	SG	9.1 ± 0.8	9.4 ± 0.9	9.3 ± 0.8

CG = control group, SG = study group.
[a]Statistically significant difference between the groups ($p < 0.05$) at the time of measurement.
Data presented as mean ± SD.
M0: baseline measurement, before induction of acute pancreatitis; M1, M2: after induction of acute pancreatitis; M3–M8: during treatment interval.

pointing toward a lesser affection to endothelial dysfunction. This is further supported by the finding of significantly higher levels of heparan sulfate at M8 in the CG compared with SG. These findings are in line with Florian et al (39) who showed that shear stress on endothelial cells reduces the heparan sulfate component of the endothelial glycocalyx and thereby directly alters the endothelial function.

In general, there are two reasons for alterations of the endothelial glycocalyx: first, the release of inflammatory mediators such as IL-6 and tumor necrosis factor-α due to inflammatory

M3	M4	M5	M6	M7	M8
865 ± 104	1,164 ± 178	1,534 ± 221	1,789 ± 260	2,140 ± 312	2,489 ± 289
686 ± 106[a]	883 ± 118[a]	1,075 ± 138[a]	1,250 ± 171[a]	1,496 ± 243[a]	1,716 ± 276[a]
224 ± 52	313 ± 131	497 ± 201	637 ± 268	786 ± 300	974 ± 326
6 ± 25[a]	25 ± 43[a]	40 ± 67[a]	76 ± 125[a]	147 ± 176[a]	220 ± 217[a]
426 ± 172	540 ± 182	704 ± 238	907 ± 304	1,107 ± 354	1,264 ± 310
461 ± 268	560 ± 288	635 ± 333	712 ± 347	821 ± 389	903 ± 419[a]
0	0.06 ± 0.21	0.04 ± 0.13	0.57 ± 1.01	0.74 ± 1.1	1.19 ± 1.45
0.42 ± 0.7[a]	0.4 ± 0.61[a]	1.1 ± 1.64[a]	1.0 ± 1.37	0.95 ± 1.03	1.53 ± 1.91
81 ± 23	79 ± 14	85 ± 19	88 ± 17	84 ± 12	92 ± 16
82 ± 12	85 ± 11	91 ± 14	91 ± 13	92 ± 13	97 ± 16
69 ± 11	64 ± 7	64 ± 7	61 ± 6	63 ± 6	63 ± 9
63 ± 6	65 ± 8	62 ± 5	61 ± 4	60 ± 4	60 ± 6
5.0 ± 2.8	4.7 ± 2.3	4.5 ± 2.2	6.2 ± 2.8	5.4 ± 2.2	5.8 ± 2.2
9.8 ± 2.9[a]	10.3 ± 3.5[a]	10.3 ± 2.7[a]	10.8 ± 3.3[a]	8.8 ± 3.0[a]	9.9 ± 3.1[a]
6.2 ± 2.8	6.3 ± 2.9	6.3 ± 2.5	7.6 ± 2.6	7.1 ± 2.5	7.7 ± 2.8
11.4 ± 3.2[a]	11.6 ± 3.0[a]	12.1 ± 3.2[a]	12.5 ± 3.5[a]	10.6 ± 3.4[a]	11.9 ± 3.5[a]
4.2 ± 2.1	3.8 ± 2.4	4.3 ± 2.7	4.1 ± 1.8	4.5 ± 2.0	5.1 ± 2.3
3.3 ± 2.2	3.1 ± 1.8	3.1 ± 2.0	3.2 ± 1.7	3.4 ± 1.3	3.2 ± 1.5[a]
55.7 ± 7.4	55.0 ± 5.0	56.1 ± 5.8	55.8 ± 9.3	55.5 ± 6.5	53.2 ± 6.2
45.3 ± 9.2[a]	45.5 ± 9.9[a]	44.0 ± 7.6[a]	44.9 ± 8.3[a]	46.7 ± 9.2[a]	43.8 ± 7.8[a]
4.5 ± 0.9	4.2 ± 0.6	4.6 ± 1.0	4.7 ± 1.0	4.5 ± 0.5	4.8 ± 1.0
3.4 ± 0.5[a]	3.6 ± 0.6[a]	3.8 ± 0.9[a]	3.9 ± 0.8[a]	4.0 ± 1.1	4.2 ± 1.2
672 ± 90	653 ± 79	662 ± 73	651 ± 100	656 ± 96	651 ± 96
564 ± 56[a]	569 ± 62	564 ± 46[a]	572 ± 56[a]	580 ± 65	608 ± 66
20.7 ± 2.2	20.4 ± 1.5	21.2 ± 1.1	20.7 ± 1.8	21.6 ± 2.2	20.5 ± 1.8
20.1 ± 3.7	20.2 ± 3.5	20.0 ± 2.9	19.7 ± 3.3	20.2 ± 3.2	20.9 ± 3.4
1,188 ± 160	1,128 ± 134	1,028 ± 187	957 ± 219	1,025 ± 139	1,010 ± 237
1,365 ± 295	1,362 ± 307 [a]	1,228 ± 232[a]	1,187 ± 231[a]	1,110 ± 270	1,121 ± 256
1.14 ± 0.37	0.93 ± 0.27	0.89 ± 0.21	0.83 ± 0.19	0.79 ± 0.24	0.78 ± 0.23
1.1 ± 0.31	0.91 ± 0.23	0.88 ± 0.2	0.85 ± 0.22	0.82 ± 0.21	0.84 ± 0.24
8.0 ± 0.7	8.4 ± 0.8	8.3 ± 1.0	8.6 ± 1.1	8.5 ± 1.0	8.4 ± 0.9
9.3 ± 0.8[a]	9.6 ± 0.9[a]	9.7 ± 1.0[a]	9.7 ± 0.8[a]	9.6 ± 0.9[a]	9.6 ± 1.0[a]

response and surgical trauma, and second, the release of atrial natriuretic peptide during iatrogenic acute hypervolemia (40, 41). These mediators are able to directly degrade the endothelial surface layer. Once this sensitive structure is destroyed, further volume application leads to an extravasation of fluids and proteins according to the classic Starling's equation, implying equalization of hydrostatic and oncotic pressures—a condition which should be avoided categorically (42). This chain of causation has been recently described (43). Our findings of elevated heparan sulfate, altered FMD, and more pancreatic

TABLE 3. Systemic and Pancreatic Tissue Oxygenation

Variable	Group	M0	M1	M2
Tissue oxygenation of the pancreas (mm Hg)	CG	54.1 ± 20.9	14.2 ± 12.6	12.7 ± 12.2
	SG	49.4 ± 17.9	17.5 ± 12.3	12.3 ± 8.3
Pao_2 (mm Hg)	CG	217 ± 52	195 ± 18	190 ± 18
	SG	197 ± 26	193 ± 18	194 ± 18
Central venous oxygen saturation (%)	CG	77.1 ± 9.5	75.2 ± 5.4	78.4 ± 6.4
	SG	75.9 ± 9	74.1 ± 10.4	75.1 ± 10.9

CG = control group, SG = study group.
[a]Statistically significant difference between the groups ($p < 0.05$) at the time of measurement.
Data presented as mean ± SD.
M0: baseline measurement, before induction of acute pancreatitis; M1, M2: after induction of acute pancreatitis; M3–M8: during treatment interval.

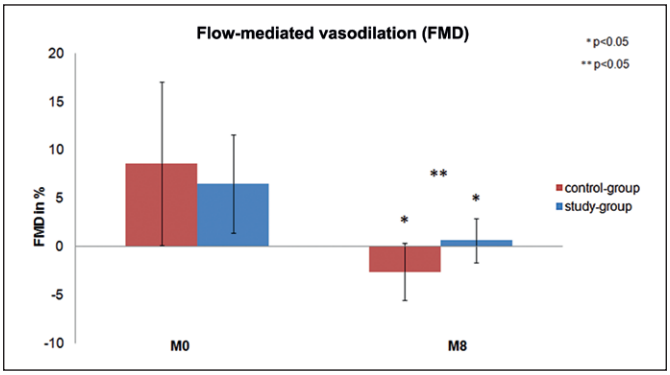

Figure 4. Variation of flow-mediated vasodilation (FMD). FMD at baseline (M0) and 6 hr after severe acute pancreatitis induction (M8). M0: baseline measurement, before induction of acute pancreatitis. M8: after treatment interval. *Statistically significant difference within the groups ($p < 0.05$) at the time of measurement (data presented as mean ± SD). **Statistically significant difference between the groups ($p < 0.05$) at the time of measurement (data presented as mean ± SD).

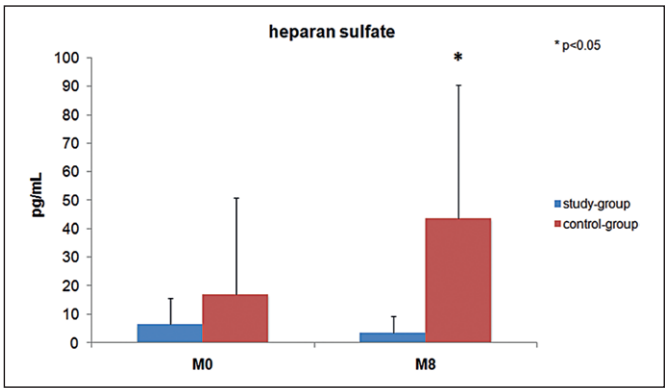

Figure 5. Serum concentrations of heparan sulfate. M0: baseline measurement, before induction of acute pancreatitis. M8: after treatment interval. *Statistically significant difference between the groups ($p < 0.05$) at the time of measurement (data presented as mean ± SD).

edema in the CG suggest that consequent maximization of SVI leads to more severe glycocalyx degradation. These aspects also might have contributed to the development of higher levels of IL-6 in the CG as a surrogate of the severity of systemic inflammation, although we found no differences in blood leukocyte count, or a higher level of acinar necrosis. This might

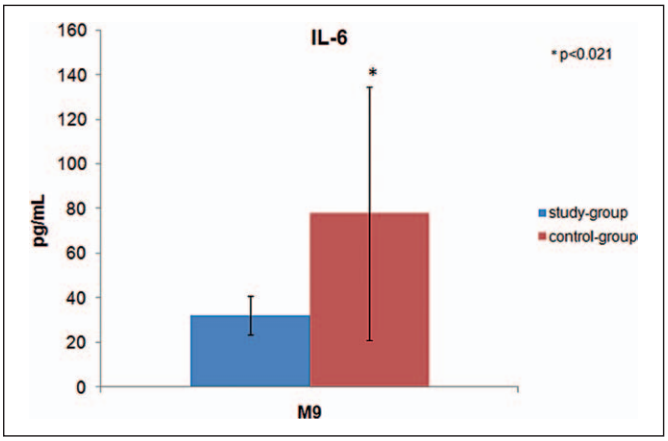

Figure 6. Serum concentrations of interleukin (IL)-6 measured on the first postoperative day (M9). *Statistically significant difference between the groups ($p < 0.05$) at the time of measurement (data presented as mean ± SD).

be explained by a higher sensitivity for IL-6 for detection of inflammation and/or by the significant higher glycocalyx damage in the CG inducing a higher IL-6 production.

Statistically significant more edema of the pancreatic head and corpus for CG indicating that volume application in situations of an altered endothelial tissue with capillary leakage leads to extravasation of this fluid.

Although there was significantly more pancreatic tissue edema in the CG, statistically significant differences in extravascular lung water index (EVLWI) could not be detected. Recent studies suggest that EVLWI is a valuable variable for evaluation of pulmonary edema, enabling realization of "overinfusion" and allowing a statement on prognosis in particular in acute respiratory distress syndrome (44, 45). Probably the overall amount of administered fluid in this study has not been enough to highlight differences in EVLWI and to induce pulmonary edema, as it was demonstrated recently by our group (7). Central venous pressure at M8, where the abdominal cavity was again closed, was moderately, but significantly, higher in the CG. This might also point toward higher intra-abdominal edema in the CG although intra-abdominal pressures could not be measured postoperatively.

M3	M4	M5	M6	M7	M8
16.4 ± 13.3	17.7 ± 13.1	16.9 ± 12.6	16.0 ± 12.3	16.5 ± 13.7	15.6 ± 11.9
13.2 ± 10.0	13.0 ± 11.0	14.3 ± 10.5	13.0 ± 9.1	15.6 ± 10.9	13.8 ± 9.2
171 ± 28	168 ± 25	156 ± 28	152 ± 36	157 ± 28	147 ± 30
188 ± 20	183 ± 20	178 ± 25[a]	172 ± 23	174 ± 30	166 ± 23
87.5 ± 5.4	85.1 ± 6.2	85.5 ± 6	85.5 ± 7.8	86.6 ± 8.5	86 ± 9.7
77.2 ± 9.5[a]	78.0 ± 9.7[a]	81.3 ± 7.6	77.3 ± 9.3[a]	77.1 ± 9.7	78.2 ± 10[a]

TABLE 4. Histopathologic Scoring of Pancreatic Tissue

Pancreatic Area	Group	Acinar Necrosis (0–3)	Fatty Tissue Necrosis (0–3)	Inflammation (0–3)	Edema (0–3)	Total (0–12)
Pancreatic head	CG	2.78 ± 0.44	1.33 ± 0.87	1.44 ± 0.88	2.89 ± 0.33	8.44 ± 1.59
	SG	2.27 ± 1.91	1.09 ± 1.14	1.18 ± 0.75	1.82 ± 0.87[a]	6.36 ± 2.77
Pancreatic corpus	CG	1.91 ± 1.3	0.64 ± 0.67	1.82 ± 0.98	2.91 ± 0.3	7.27 ± 1.35
	SG	2.3 ± 1.06	0.5 ± 0.53	1.6 ± 0.7	2.2 ± 0.92[a]	6.7 ± 2.21
Pancreatic tail	CG	1.9 ± 1.37	0.5 ± 0.53	1.9 ± 1.0	2.5 ± 0.53	6.8 ± 1.87
	SG	2.33 ± 1.12	0.6 ± 0.52	1.6 ± 1.08	2.3 ± 0.82	6.5 ± 2.32

CG = control group, SG = study group.

[a]Statistically significant difference between the groups ($p < 0.05$).

Data presented as mean ± SD.

Histopathologic score for severe acute pancreatitis. Histopathological score for the pancreatic head, corpus, and tail: 0 (no pancreatitis) to 3 (severe pancreatitis). In total score: 0 (no pancreatitis) to 12 (severe pancreatitis).

Some methodological weaknesses need to be taken into consideration. First, this study was an animal study, and although pigs are used as the best and most common model in macrohemodynamic studies, results should not be transferred to clinical practice unquestioned. Nonetheless, the model used closely represents the clinical situation of acute biliary pancreatitis, caused by obstruction of the papilla by a stone, and has previously been well established (11). However, for reasons of the experimental setup, unfortunately, we lack data on the long-term course. Furthermore, again for reasons of experimental setup, all animals were mechanically ventilated which will not be the case with every patient suffering from SAP, especially in the early phase. Hence, in this study, we used SVI, which is independent of mechanically ventilation, as treatment goal. To guide fluid therapy, we defined an individual physiologic SVI. This was prior to SAP induction and after induction of anesthesia. This of course is not directly transferable to clinical setting, but this was necessary owing to the experimental setup and allowed to principally challenge the formulated hypothesis of an "individual SVI" as treatment target. To define the correct "individual target SVI" will be one of the main challenges when converting this concept into clinical practice and to different patient groups. Of course, the definition of this individual hemodynamic optimum remains one of the main limitations of the hypothesis raised by this feasibility study. It is already difficult to define an optimal SV since preexisting illness, such as cardiac disease, will all have a tremendous impact on the individual SV. Thus, in further research also functional variables of oxygen delivery and consumption, such as $Scvo_2$, and metabolic variables, such as lactate and lactate clearance, need to be included to realize such an approach to individual "normal" values.

We used a polarographic measuring probe (Licox GMS, Kiel, Germany) to gauge the tissue oxygenation in the pancreas. This technique is just an evidence to quantify tissue oxygenation, but no direct evaluation of microcirculatory by flux measuring (46).

Further, for fluid resuscitation, we used Ringer solution and hydroxyethyl starch 6% in a fixed ratio of 1:1. Currently, the use of starches in patients with sepsis is an ongoing controversial discussion. Indications of colloid administration have to be regarded critically, distinguishing between an initial volume resuscitation phase (6 hr) and fluid maintenance phase (47–50).

For the FMD, endothelium-independent vasodilatation was not evaluated by sublingual nitrate administration because of the contemporaneous elevation of hemodynamic variables and to minimize effects on hemodynamic therapy. Finally, it is

difficult to conclusively separate the effect of vasoactive drugs on FMD evaluation.

CONCLUSIONS

Individualized optimization of hemodynamics oriented on personalized physiological goals of SVI as strategy for hemodynamic stabilization during the early course of SAP leads to less vascular endothelial damage, inflammatory response, and less pancreatic edema when compared to a treatment strategy of maximizing SV.

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