Original Article

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Serum markers for early detection of patients with mesenteric ischemia after cardiac surgery

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Abstract

Objective: Mesenteric ischemia (MESI) is a rare but often fatal complication in patients after cardiac surgery. Non-specific clinical symptoms and lack of specific laboratory parameters complicate the diagnosis. We evaluated potential serum markers for MESI in cardiac surgery patients.

Methods: Between March and October 2012, serial serum samples of 567 elective cardiac surgery patients were collected 1, 24, and 48 h after the operation, and concentrations of potential markers for MESI [α-glutathione-S-transferase (αGST), intestinal fatty-acid-binding protein (iFABP), and D-lactate] were measured retrospectively. In patients requiring laparotomy, blood samples obtained 72, 48, 24, and 12 h before the laparotomy were additionally measured and compared to all other patients (control group).

Results: Laparotomy was performed in 18 patients at 11 ± 7 days after cardiac surgery. MESI was found in 9/18 patients. Already 1 h after cardiac surgery, the serum concentrations of D-lactate (37 ± 18 vs. 25 ± 20 nmol/mL, $p < 0.01$) and αGST (82 ± 126 vs. 727 ± 1382 μg/L, $p < 0.01$) in patients undergoing laparotomy were increased compared to the control group. Between patients with and without MESI, differences were only found for iFABP 24 h after cardiac surgery (1.1 ± 0.4 vs. 2.9 ± 0.6 ng/mL, $p = 0.04$) and up to 72 h before laparotomy (0.56 ± 0.72 vs. 2.51 ± 1.96 ng/mL, $p = 0.01$).

Conclusions: D-lactate and αGST were early markers for gastrointestinal complications after cardiac surgery. Before laparotomy, lowered iFABP levels indicated MESI. Routinely used, these markers can help identify patients with gastrointestinal complications after cardiac surgery early, and might be useful for the evaluation of new therapeutic or preventive strategies.

Keywords: αGST; biomarker; cardiac surgery; D-lactate; iFABP; mesenteric ischemia.

Dedicated to: the memory of Prof. Dr. Dr. Herbert de Groot who died unexpectedly and much too early on May 10, 2016.

Introduction

Mesenteric ischemia (MESI) is a rare complication in patients after cardiac surgery, with an incidence of 0.1–0.5% and high mortality rates between 60% and 100% [1–3].

The pathophysiological mechanisms for MESI after cardiac surgery are multimodal. It is well known that cardiopulmonary bypass (CPB) causes systemic inflammation [4], reduction of intestinal perfusion [5], microcirculatory alterations, loss of intestinal barrier function [6], and hemolysis [7]. However, postoperatively low cardiac output, intravascular volume deficiency, and the need for vasopressors restrict mesenteric perfusion. Unfortunately, mesenteric malperfusion itself is a trigger of all the above-mentioned factors, resulting in a *circulus vitiosus* process.

The diagnosis of MESI after cardiac surgery is difficult in the setting of mostly sedated, intubated patients, with low cardiac output, systemic inflammatory response

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syndrome, need for vasopressors, and usually many differential diagnoses for acute abdomen. Early diagnosis and surgical treatment have been suggested to be the only beneficial factors for the prognosis of these patients [2, 8, 9]. Recent publications indicated that different biomarkers have some impact on the detection of MESI [10–12]. Indeed, α-glutathione-S-transferase (αGST), intestinal fatty-acid-binding protein 2 (iFABP-2), and D-lactate are the most promising biomarkers for the early and accurate detection of MESI. These biomarkers are explained in detail below.

First, the GSTs are a family of enzymes involved in the detoxification of a range of toxic and foreign compounds within the cell by conjugation to glutathione, although different isoforms exist, such as α, π, θ, and μ, which are distributed in relatively specific organs [13]. For example, αGST is a unique biomarker for hepatic and intestinal injuries.

Second, FABPs display a high affinity for long-chain fatty acids and seem to function in metabolism and intracellular transport of lipids. Three distinct FABPs are known: liver FABP, iFABP, and ileal lipid binding protein distributed proximally to distally in the intestine. The iFABP is detectable along the entire length of the small intestine and is maximally represented near the medial segment [14].

Finally, D-lactate and L-lactate result from the reduction of pyruvate by D- and L-lactate dehydrogenases, respectively. These enzymes uniquely exist in bacteria or mammals, respectively. Therefore, the quantification of D-lactate has been revealed as a marker for bacterial translocation, which follows intestinal or colonic mucosal injury caused by ischemia or other reasons [15].

Therefore, the aims of this study were (i) to quantify and monitor in a time-dependent manner these markers in patients after cardiac surgery and (ii) to evaluate their potential in identifying patients at a risk for MESI.

Methods

Patient population and characteristics

Between March and October 2012, a total of 567 elective consecutive cardiac surgery patients were included in this study. Institutional Ethics Committee review and approval for this prospective study were obtained (12-5066-BO), and patients gave written informed consent. Patient-specific parameters were stored anonymized in a project-specific internal database. The mean age was 67 ± 12 years, and 393/567 (69%) of the patients were male. Of the 567 patients, 495 (87.3%) were operated using CPB. The procedures performed with and without CPB, and the comorbidities are listed in Table 1.

Table 1: Procedures and comorbidities of patients operated with and without cardiopulmonary bypass (CPB), coronary artery bypass grafting (CABG), aortic valve repair/replacement (AVR), mitral valve repair/replacement (MVR), ventricular assist device (VAD), off-pump coronary artery bypass grafting (OPCAB), transcatheter aortic valve replacement (TAVR), arterial hypertonus (aHTN), diabetes mellitus (DM), chronic obstructive pulmonary disease (COPD), and chronic atrial fibrillation (Chron. aFib).

Operative procedures and comorbidities	Number (%), 567 (100)	
With CPB		
	495	(87.3)
AVR	35	(6.2)
CABG	242	(42.7)
MVR	25	(4.4)
Aortic	21	(3.7)
Combination	154	(27.2)
VAD/transplant	16	(2.8)
Others	12	(2.1)
Without CBP		
	72	(12.7)
OPCAB	5	(0.9)
TAVR	40	(7.1)
Others	17	(3.0)
Comorbidities		
aHTN	483	(85.2)
DM	162	(28.6)
COPD	152	(26.8)
Active smoking	107	(18.9)
pAVD	94	(16.6)
BMI >30	150	(26.5)
NYHA ≥3	458	(80.8)
Chron. aFib	87	(15.3)
Previous stroke	64	(11.3)
Dialysis	32	(5.6)

BMI, body mass index; NYHA, New York Heart Association; pAVD, peripheral arterial vascular disease.

Study protocol and analyzed serum samples

The remaining volume of all routinely taken blood samples in the intensive care unit (ICU) from all included cardiac surgery patients was aliquoted and stored at −80 °C directly after measurement of the routine parameters, which avoided the need for additional blood sampling in the interest of the patient. Patients were routinely treated according to the internal ICU protocols. Due to the high technical effort and considerable costs for the enzyme-linked immunosorbent assay (ELISA) and enzyme assays, αGST, iFABP-2, and D-lactate were measured retrospectively at the end of the study period in all available blood samples taken 1, 24, and 48 h after the cardiac surgery. In patients with laparotomy blood samples obtained 72, 48, 24, and 12 h before laparotomy, the markers were additionally measured retrospectively. Patients with laparotomy with and without MESI were compared to all other patients, defined as the control group.

In 57/567 (10%) patients, no serum samples were taken within the first postoperative hour; 65/567 patients were discharged from the ICU within the first 24 h, and 254/567 (45%) patients within the first

48 h. Serum samples taken 1, 24, and 48 h after cardiac surgery in 510 (90%), 502 (89%), and 313 (55%) patients, respectively, and up to 72 h before laparotomy in 18 patients were analyzed.

Biomarkers

The serum concentrations of iFABP-2 and αGST were analyzed using a commercially available ELISA kit (iFABP-2: Hölzel-Biotech, Köln, Germany; αGST: Otto Nordwald GmbH, Hamburg, Germany) according to the manufacturer's recommendations. D-lactate was measured by a colorimetric enzyme assay (BioCat Biovision, Heidelberg, Germany) according to the manufacturer's recommendations.

Data analysis and statistics

Data were collected prospectively and analyzed retrospectively. Statistical analysis and figures were done using GraphPad Prism version 7.0a for Mac (GraphPad Software, La Jolla, CA, USA) and Stata statistical software (version 11.2; StataCorp, College Station, TX, USA). Due to the small group size, continuous variables were compared by the Wilcoxon signed-rank test or t-test, with the α level set at 0.05 for statistical significance.

Results

MESI after cardiac surgery is associated with high mortality

Laparotomy due to expected MESI or clinical signs of an acute abdomen was performed in 18/567 (3.2%) patients after 11 ± 7 days of cardiac surgery. The indication for laparotomy was primarily given based on clinical signs of an acute abdomen, continuous hyperlactatemia, or increasing need for vasopressors. Additional computed tomography imaging without evidence of any occlusive mesenteric malperfusion was performed in 7/18 patients. Angiography was performed in one patient. In 9/18 (50%) patients, MESI was found in the ischemic mesenteric segment. The sigma and colon were affected in 7/9 (78%) cases, and the small intestine in 2/9 (22%) cases. Histology revealed transmural necrosis in 5/9 (56%) patients and submucosal necrosis in 4/9 (44%) patients. In patients without resection, no abdominal pathology was found in 5/9 (56%) cases, paralytic ileus in 2/9 (22%) cases, as well as one obscure non-necrotic small intestine perforation and one necrotic gallbladder. Mortality occurred in 8/9 (89%) patients in the laparotomy group with MESI and in 6/9 (67%) patients in the laparotomy group without MESI.

Patients with laparotomy during the postoperative course were found to have significantly more cardiovascular risk factors with a higher rate of diabetes (61% vs. 27%, $p = 0.002$), more smoking (50% vs. 18%, $p < 0.001$), and more peripheral artery disease (61% vs. 15%, $p < 0.001$). Furthermore, patients with laparotomy during the postoperative course had longer bypass times (151 ± 74 vs. 120 ± 55 min, $p = 0.023$) with CPB time >150 min in 67% vs 21% of the patients ($p < 0.001$). The rate of patients with need for inotropic support already before cardiac surgery was higher in the laparotomy group (38.9% vs. 3.5%, $p < 0.001$). Also, postoperative atrial fibrillation (12% vs. 28%, $p = 0.043$) and heart failure (8.6% vs. 39%, $p < 0.001$) were more frequent in patients requiring laparotomy later. Laparotomy patients demonstrated significantly higher gastrointestinal complication scores (GICS) (12.97 vs. 3.13, $p < 0.001$) and EuroSCORE II scores (30.75 vs. 6.6, $p < 0.001$).

Markers after cardiac surgery and before laparotomy

Within the first 48 h after cardiac surgery, significantly higher αGST and D-lactate serum concentrations were found in patients with laparotomy compared to patients without laparotomy. The iFABP serum concentration was significantly lower in the laparotomy group 1 h after cardiac surgery.

Comparing laparotomy patients with and without MESI up to 3 days before laparotomy, no significant differences between these two groups were found for αGST and D-lactate, but the iFABP-2 levels were significant lower in the MESI group.

High αGST levels are associated with laparotomy

Already 1 h after cardiac operation, nearly 10-fold higher αGST concentrations were found in the serum of patients needing a laparotomy 11 ± 7 days later compared to patients without laparotomy. After 24 h, the αGST concentrations in the laparotomy group were >25-fold higher compared to the control group and remained significantly higher after 48 h (Table 2, Figure 1A). However, the differences in αGST serum concentration between the laparotomy patients with and without MESI were not significant within 48 h after cardiac surgery (Table 2, Figure 2A). Up to 3 days before laparotomy, the αGST levels in MESI and non-MESI patients did not differ significantly but were

Table 2: Mean values ± standard deviation (SD) of αGST, D-lactate, and iFABP 1, 24, and 48 h after cardiac surgery for the control group and laparotomy group with subgroup analysis for MESI and non-MESI patients.

	Control, mean ± SD (n)	Laparotomy, mean ± SD (n)	p-Value	MESI, mean ± SD (n)	Non-MESI, mean ± SD (n)	p-Value
αGST (µg/L)						
1 h	82 ± 126 (492)	727 ± 1382 (18)	<0.01	280 ± 245 (9)	1173 ± 1884 (9)	0.35
24 h	73 ± 167 (484)	4549 ± 9889 (18)	<0.01	3741 ± 8542 (9)	1459 ± 3013 (9)	0.63
48 h	83 ± 153 (295)	2300 ± 5895 (18)	<0.01	1010 ± 1710 (9)	3204 ±8282 (9)	0.63
D-lactate (nmol/mL)						
1 h	25.1 ± 20.1 (492)	37.1 ± 17.9 (18)	<0.01	36.3 ± 20.8 (9)	37.8 ± 15.9 (9)	0.51
24 h	23.2 ± 21.1 (484)	52.2 ± 40.5 (18)	<0.01	58.7 ± 40.9 (9)	45.7 ± 41.4 (9)	0.23
48 h	24.8 ± 23.6 (295)	58.7 ± 54.3 (18)	<0.01	56.3 ± 44.6 (9)	61.1 ± 65.2 (9)	0.69
iFABP (ng/mL)						
1 h	4.05 ± 4.04 (492)	1.04 ± 0.91 (18)	<0.01	0.78 ± 0.71 (9)	1.30 ± 1.05 (9)	0.29
24 h	3.42 ± 4.95 (484)	2.03 ± 1.86 (18)	0.38	1.15 ± 1.30 (9)	2.90 ± 1.99 (9)	0.04
48 h	4.28 ± 6.12 (295)	2.88 ± 2.66 (18)	0.95	1.89 ± 1.78 (9)	3.87 ± 3.11 (9)	0.12

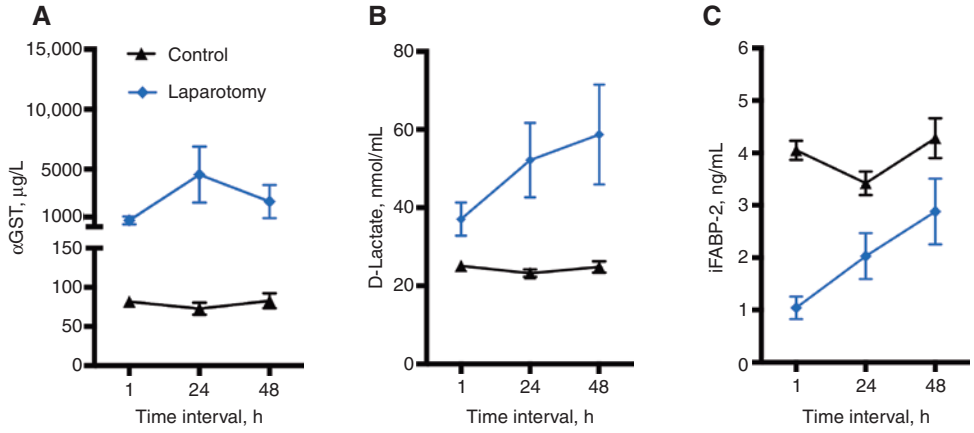

Figure 1: Already 1 h after cardiac surgery and 24 and 48 h thereafter, the serum concentrations of D-lactate and αGST in patients undergoing laparotomy were increased compared to the control group.
Timeline of αGST (A), D-lactate (B), and iFABP (C) mean serum concentrations and 95% confidence interval (CI) at 1, 24, and 48 h after cardiac surgery for the control group and the laparotomy group.

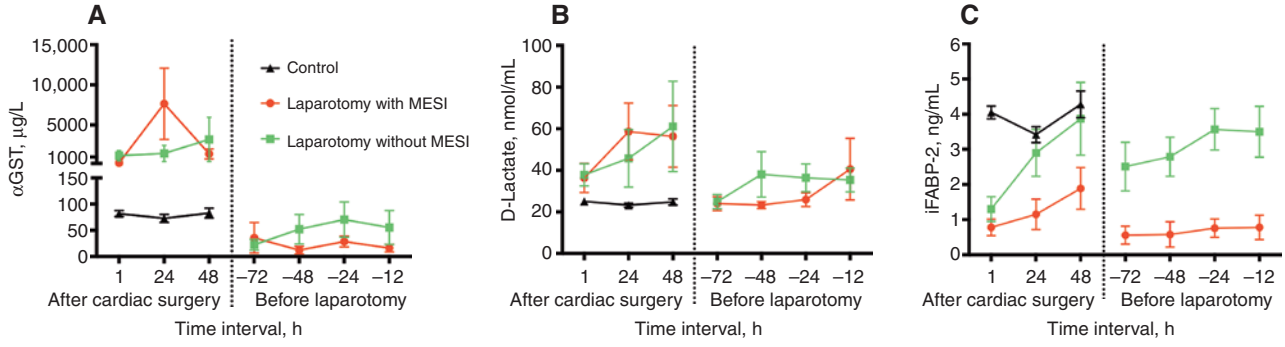

Figure 2: Comparing laparotomy patients with and without MESI up to 3 days before laparotomy, no significant differences between these two groups were found for αGST and D-lactate, but the iFABP-2 levels were significant lower in the MESI group.
Timeline of αGST (A), D-lactate (B), and iFABP (C) mean serum concentrations and 95% CI at 1, 24, and 48 h after cardiac surgery for the control, MESI, and non-MESI group at 1, 24, and 48 h after cardiac surgery and 72, 48, 24, and 12 h before laparotomy.

Table 3: Mean serum concentration ± SD of αGST, D-lactate, and iFABP 72, 48, 24, and 12 h before laparotomy for MESI and non-MESI patients. One patient underwent laparotomy 48 h after cardiac surgery.

	MESI, mean ± SD (n)	Non-MESI, mean ± SD (n)	p-Value
αGST (μg/L)			
– 72 h	35.8 ± 82.4 (8)	22.6 ± 29.1 (8)	0.40
– 48 h	12.3 ± 20.6 (8)	52.1 ± 79.8 (8)	0.29
– 24 h	28.6 ± 30.9 (9)	70.6 ± 100.5 (9)	0.55
– 12 h	16.3 ± 21.6 (9)	55.4 ± 96.7 (9)	0.77
D-lactate (nmol/mL)			
– 72 h	24.1 ± 9.5 (8)	24.9 ± 9.6 (8)	0.83
– 48 h	23.3 ± 4.7 (8)	38.1 ± 30.9 (8)	0.34
– 24 h	25.9 ± 9.8 (9)	36.4 ± 19.9 (9)	0.09
– 12 h	40.6 ± 41.8 (9)	35.4 ± 17.1 (9)	0.63
iFABP (ng/mL)			
– 72 h	0.56 ± 0.72 (8)	2.51 ± 1.96 (8)	0.01
– 48 h	0.58 ± 1.02 (8)	2.79 ± 1.57 (8)	0.01
– 24 h	0.76 ± 0.78 (9)	3.57 ± 1.77 (9)	<0.01
– 12 h	0.78 ± 1.04 (9)	3.50 ± 2.17 (9)	0.01

even lower than the initial postoperative levels of the control group (Table 3, Figure 2A).

High D-lactate levels are associated with laparotomy

Significantly higher D-lactate concentrations were found in the laparotomy group compared to patients without laparotomy 1 h after cardiac surgery. Within the next 48 h, these differences amplified and D-lactate levels were more than twice as high in the laparotomy group (Table 2, Figure 1B). No significant differences between the laparotomy patients with and without MESI were found within the first 48 h after cardiac surgery (Table 2, Figure 2B). Before laparotomy, no significant differences between laparotomy patients with and without MESI were found. The measured D-lactate levels 72, 48, 24, and 12 h before laparotomy were comparable to the levels measured in the control group after cardiac surgery (Table 3, Figure 2B).

Low iFABP levels are associated with MESI

Only 1 h after cardiac surgery, serum iFABP concentrations between laparotomy and non-laparotomy patients differed significantly. The iFABP concentrations were lower by a factor of 4 in the laparotomy group. Within the next 48 h, the iFABP concentrations remained lower in the laparotomy group, but not significantly (Table 2, Figure 1C). Laparotomy patients with and without MESI both had similarly low iFABP-2 serum concentrations 1 h after cardiac surgery. After 24 h, MESI patients had significantly lower iFABP serum concentrations. However, the iFABP-2 levels of laparotomy patients without MESI were similar to those of patients without laparotomy after 24 and 48 h (Table 2, Figure 2C). Before laparotomy, significant differences between laparotomy patients with and without MESI were found. Already 72 h before laparotomy, MESI patients had significantly lower iFABP-2 levels compared to laparotomy patients without MESI. These differences remained significant 48, 24, and 12 h before laparotomy (Table 3, Figure 2C).

Discussion

The incidence of MESI after cardiac surgery in the literature varies. In a review with 35 included papers and a total of 151,652 patients, Rodriguez et al. calculated an incidence of 0.16% with 58% mortality [1]. Pang et al. described an even higher incidence (0.31%) and mortality (71%) [2], and retrospective autopsy studies found MESI in 0.49% of the patients, responsible for 11% of all postoperative deaths [3]. Interestingly, 96% of these had a non-occlusive MESI (NOMI), which is interesting regarding the definition and therapy of MESI, which will be addressed later. Our high laparotomy and MESI rate of 1.6% might be the result of a bias during the study period; however, it reflects the severely ill patient population with a mean EuroSCORE I of 11 ± 14%. According to the GICS model introduced by Nilsson et al. [16], the expected MESI rate in our patient population is >1.9%, emphasizing the accuracy of the suggested score and the frailty of the observed patient cohort.

In the setting of cardiac and aortic surgery, some studies focused on αGST, D-lactate, and iFABP, and their elevation during operations with and without CPB [17–19]. On-pump coronary artery bypass grafting (CABG) patients showed significantly higher serum αGST levels at the end of surgery (10 μg/L) compared to off-pump CABG patients (4 μg/mL), normalizing 24 h after surgery [17]. Although the reported mean values are low compared to our total control group, they are similar to the mean values of an uncomplicated CABG subgroup (6 μg/mL), demonstrating the general impact of CPB on mesenteric perfusion and intestinal barrier function. In another study focusing on intestinal barrier function, Sun et al. found the highest D-lactate concentration (11 nmol/mL) 2 h postoperatively

and the highest iFABP concentration (1.4 ng/mL) 6 h postoperatively in aortic valve repair/replacement patients. Both findings and values are conclusive with the results of our control group, again emphasizing damage and loss of intestinal barrier function during any operation using CPB. Also, in patients operated for abdominal aortic aneurysm (AAA), elevated D-lactate levels (33 vs. 11 nmol/mL [20]) were found in patients with histologically diagnosed ischemic colitis 24 and 48 h after open aortic reconstruction. Patients with a fatal intestinal necrosis after AAA repair had increased iFABP levels (0.6 vs. 4 ng/mL) early postoperatively and decreased concentrations on postoperative days 3 and 4 [21].

D-lactate and αGST showed no differences between patients with and without MESI, neither 72, 48, 24, or 12 h before laparotomy nor early after cardiac surgery. However, both markers were significantly increased in the laparotomy group within the first 2 days after cardiac surgery, suggesting a pathologic gastrointestinal process 11 ± 7 days before clinical signs or imaging indicated laparotomy.

Interestingly, in our study, iFABP-2 was decreased in the laparotomy group compared to the control group early after cardiac surgery and increased again within the next 48 h, although the iFABP-2 concentration remained significantly lower in the MESI subgroup compared to the non-MESI subgroup. Before laparotomy, MESI patients had significantly lower iFABP-2 levels compared to non-MESI patients. These findings might be explained by our definition of MESI, which was a necrotic intestine diagnosed by laparotomy. In a human model, Schellekens et al. demonstrated the reduction of tissue and serum iFABP-2 within 120 min reperfusion after up to 60 min ischemia by subtotal destruction of the intestinal enterocytes and villus structures [22]. In histology of the resected intestinal segments in our study, transmural necrosis was found in 5/9 patients, and submucosal necrosis in 4/9 patients. In all specimens, no mucosa that could have released iFABP-2 was found.

The definition of MESI as intestinal ischemia treated by laparotomy and resection is unambiguous and used in most retrospective studies. However, it does not reflect the dynamics of this disease and its pathomechanism, which is non-occlusive (NOMI) in 95% [3] with an incidence after cardiac surgery of up to 10% [23]. We assume an even higher rate of mild and non-apparent MESI after most cardiac operations, demonstrated by elevated markers after CPB in all studies including ours. αGST and D-lactate seem to be suitable markers for early detection of gastrointestinal complications after cardiac surgery, and iFABP-2 could help differentiate patients with ischemia. Especially in high-risk patients with CPB times >150 min, pre- and postoperative need for inotropic support, atrial fibrillation, and high GICS and EuroSCORE, these markers might be used for differentiation. Nevertheless, resection of necrotic tissue is only damage control, and the benefit of a very early resection is questionable. Accepting the pathologic mechanism and the dynamic character, new therapeutic and protective approaches are required. Substances like glycine [24, 25] and pyruvate [26] demonstrated protective effects in MESI models in rats. The evaluated markers could not only be useful for early detection of patients with MESI, but also represent a new routine for evaluation of protective effects of different substances or conditioning maneuvers in further clinical studies.

Author Statement

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Authors Contributions

Study conception and design: DSD, HJ, FP and HdG; Acquisition of data: DSD, CB and KHS; Analysis and interpretation of data: DSD, CB, HdG and AC; Drafting of manuscript: DSD, CB and MW Critical revision: AC, FP, HJ and KT.

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Supplementary Material: The article (https://doi.org/10.1515/iss-2018-0035) offers reviewer assessments as supplementary material.

Reviewer Assessment

Daniel-Sebastian Dohle*, Carolin Bestendonk, Frank Petrat, Konstantinos Tsagakis, Meng Wang, Karl-Heinz Strucksberg, Ali Canbay, Heinz Jakob and Herbert de Groot

Serum markers for early detection of patients with mesenteric ischemia after cardiac surgery

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Reviewers' Comments to Original Submission

Reviewer 1: Theodosios Bisdas

Oct 14, 2018

Reviewer Recommendation Term:	Accept with Minor Revision
Overall Reviewer Manuscript Rating:	75

Custom Review Questions	**Response**
Is the subject area appropriate for you?	3
Does the title clearly reflect the paper's content?	5 - High/Yes
Does the abstract clearly reflect the paper's content?	5 - High/Yes
Do the keywords clearly reflect the paper's content?	5 - High/Yes
Does the introduction present the problem clearly?	5 - High/Yes
Are the results/conclusions justified?	4
How comprehensive and up-to-date is the subject matter presented?	4
How adequate is the data presentation?	3
Are units and terminology used correctly?	3
Is the number of cases adequate?	3
Are the experimental methods/clinical studies adequate?	4
Is the length appropriate in relation to the content?	4
Does the reader get new insights from the article?	4
Please rate the practical significance.	3
Please rate the accuracy of methods.	4
Please rate the statistical evaluation and quality control.	4
Please rate the appropriateness of the figures and tables.	5 - High/Yes
Please rate the appropriateness of the references.	4
Please evaluate the writing style and use of language.	5 - High/Yes
Please judge the overall scientific quality of the manuscript.	4
Are you willing to review the revision of this manuscript?	Yes

Comments to Authors:

1. Methods:
a) Do you think that the retrospective measurement of all markers in the blood samples could bias the results? Where and how did you preserve the samples?
b) When you are talking about MESI, do you include the non-occlusive type? How did you recognise NOMI? Have you performed a CT before laparotomy and if yes, could you provide more information about the etiology of the MESI?
c) Statistical analysis: I can imagine that you used Wilcoxon in case of samples coming from the same patient. Which test was used when you compared patients with lapartomy with and without MESI?
2. Results:
a) Please provide the rates always (e.g. 18/567: 3%)
b) How many of those patients developing MESI or with the clinical suspicion of MESI were treated on CPB? Moreover, what kind of operations were performed and did you observe any intraoperative complications or prolonged op time in this group?
c) Could you provide any comparisons between patients with NOMI or non-NOMI?
d) How did you treat the patients with MESI?
e) Could you provide a table with the type of operations in patients with laparotomy?
3) Discussion:
a) Are these outcomes going to change sth in your daily practice?
b) Why should you perform a laparotomy and not a laparoscopy first?
4. General comments:
A) The topic of your research is very important and interesting. However, the presentation of the cohort is a little bit difficult. You have to explain the type of MESI; if all cases were NOMI, then you have to change the terminology.
B) Try to determine if there was a correlation between type of operation, op time and ICU stay with the need of laparatomy or MESI. By this way, you can recommend that in these types of patients (e.g. patient after mitral valve replacement with op duration > 4h, and ICU stay > 2 days) you have to check these markes. This would be very helpful for the readers.
C) You are talking about early diagnosis but it seems that this will not influence the outcome according to the literature in case of NOMI. Did I understand it right? In this case, you have to discuss the real benefit of early detection.

Reviewer 2: anonymous

Oct 09, 2018

Reviewer Recommendation Term:	Reject
Overall Reviewer Manuscript Rating:	20
Custom Review Questions	**Response**
Is the subject area appropriate for you?	3
Does the title clearly reflect the paper's content?	3
Does the abstract clearly reflect the paper's content?	3
Do the keywords clearly reflect the paper's content?	3
Does the introduction present the problem clearly?	3
Are the results/conclusions justified?	2
How comprehensive and up-to-date is the subject matter presented?	4
How adequate is the data presentation?	2
Are units and terminology used correctly?	4
Is the number of cases adequate?	1 - Low/No
Are the experimental methods/clinical studies adequate?	2
Is the length appropriate in relation to the content?	3
Does the reader get new insights from the article?	1 - Low/No
Please rate the practical significance.	2
Please rate the accuracy of methods.	3
Please rate the statistical evaluation and quality control.	2
Please rate the appropriateness of the figures and tables.	3
Please rate the appropriateness of the references.	2
Please evaluate the writing style and use of language.	4
Please judge the overall scientific quality of the manuscript.	2
Are you willing to review the revision of this manuscript?	Yes

Comments to Authors:

The presented work “Serum Markers for early Detection of Patients with Mesenteric Ischemia after Cardiac Surgery” deals with the important question if there are markers which can predict a mesenteric ischemia in cardio-surgical patients.
However, the quality of data acquisition is poor and the paper has several major flaws:
- there was a similar study published by Ludewig et al. in 2017. Why was this study not cited or the results discussed since they are completely different to the results of the presented study?
- how was the decision for performing a laparotomy made? CT-based? Clinical state?
- the number of patients is too low to draw a significant conclusion. Only 1,5 % of the observed patients received a laparotomy, the study design seems to be a random shot.
- IFABP is mainly produced in the small intestine, but only two patients had a small bowel ischemia. How do the authors interpret this?
- How many patients needed a perioperative renal replacement therapy?
- How many patients suffered from liver cirrhosis?

Reviewer 3: anonymous

Oct 25, 2018

Reviewer Recommendation Term:	Accept with Minor Revision
Overall Reviewer Manuscript Rating:	50

Custom Review Questions	**Response**
Is the subject area appropriate for you?	3
Does the title clearly reflect the paper’s content?	5 - High/Yes
Does the abstract clearly reflect the paper’s content?	4
Do the keywords clearly reflect the paper’s content?	4
Does the introduction present the problem clearly?	5 - High/Yes
Are the results/conclusions justified?	3
How comprehensive and up-to-date is the subject matter presented?	3
How adequate is the data presentation?	3
Are units and terminology used correctly?	3
Is the number of cases adequate?	2
Are the experimental methods/clinical studies adequate?	4
Is the length appropriate in relation to the content?	4
Does the reader get new insights from the article?	3
Please rate the practical significance.	2
Please rate the accuracy of methods.	3
Please rate the statistical evaluation and quality control.	3
Please rate the appropriateness of the figures and tables.	4
Please rate the appropriateness of the references.	4
Please evaluate the writing style and use of language.	4
Please judge the overall scientific quality of the manuscript.	3
Are you willing to review the revision of this manuscript?	Yes

Comments to Authors:

This is a nice paper which describes the difficulty of discover a mesenteric ischemia after cardiac surgery. There are no standard clinical markers after surgery. Normally it is a combination of clinical examination an high serum lactat. But lactat after cardiac surgery is often high because of the low cardiac output after cardiac surgery in patients with preoperativ low ejection fraction (EF). This results in a relative ischemia with higher serum lactat. Most mesenteriac ischemia are non occlusive so diagnostic with CT scan shows the extent of mesenteriac ischemia just in an advaced stage, when a surgery is almost to late. Non occlusive MI is often a result of high catecholamines in patients with low cardiac Output, so it is a circulus vitiosus. The idea of finding markers for MESI is really important and make the decision for a laparotomy in patients after cardiac surgery easier.
I would be interested in the number of patients in your study who died because of MI after cardiac surgery and were not presented for surgery?

Authors' Response to Reviewer Comments

Oct 30, 2018

Reviewer#1

1. Methods:

Question#a: Do you think that the retrospective measurement of all markers in the blood samples could bias the results? Where and how did you preserve the samples?

Answer#a: In fact, the retrospective measurement of the samples could change the actual value. The samples were stored at -80°C after centrifugation and collected the regular laboratory parameters and then measured in larger groups to have sufficient samples for the rather expensive essays. In preliminary experiments with animal samples, the duration of the sample storage had no influence on the measurement result. If, however, a non-excludable error should have occurred due to the time interval until storage, this would be a systematic error that should not have led to any bias.

Question#b: When you are talking about MESI, do you include the non-occlusive type? How did you recognise NOMI? Have you performed a CT before laparotomy and if yes, could you provide more information about the etiology of the MESI?

Answer#b: In our opinion, mesenteric ischemia (MESI) is the final stage of a mesenteric underperfusion either on the basis of an occlusive or non-occlusive (NOMI) cause. In order to have the hardest possible endpoint, we used the laparatomy and the intraoperative result of this. In 7/18 patients a CT and one angiography was performed before laparotomy, without any evidence of occlusion of an intestinal vessel. In most cases, however, the intestinal vessels were narrow and reduced in perfusion. However, since the CT findings were rarely unambiguous, laparotomy was performed in conjunction with the clinical signs. (We added the sentences: "Indication for laparotomy was primarily given by clinical signs for an acute abdomen, continuous hyperlactatemia or increasing need for vasopressors. Additional CT-imaging without evidence for any occlusive mesenteric malperfusion was performed in 7/18 patients. Angiography was performed in one patient") In all cases, as in cardiosurgical patients typical, MESI was due to a low cardiac output and high catecholamines doses leading to NOMI. As described in the literature, we believe that a NOMI of varying magnitude occurs in almost every patient undergoing cardiac surgery, but can be compensated in most patients, and leads to a final image of MESI in only a few patients.

Question#c: Statistical analysis: I can imagine that you used Wilcoxon in case of samples coming from the same patient. Which test was used when you compared patients with lapartomy with and without MESI?

Answer#c: For statistical analysis of this paper we worked with an professional statistician, who we contacted again. The patient with and without MESI were compared by an simple t-test. This was added to the statistical part (change was marked red)

2. Results:

Question#a: Please provide the rates always (e.g. 18/567: 3%)

Answer#a: Rates were changed and marked red.

Question#b: How many of those patients developing MESI or with the clinical suspicion of MESI were treated on CPB? Moreover, what kind of operations were performed and did you observe any intraoperative complications or prolonged op time in this group?

Answer#b: In all but one patient with subsequent laparotomy (thoracoabdominal aortic replacement with axillo-femoral bypass) the heart lung machine was used. The types of surgery in the laparotomy group were: two isolated coronary bypass operations, one biological aortic valve replacement, 2 left ventricular assist devices, 3 lung trans pantations, 9 combination procedures, and one thoracoabdominal replacement. The mean Euroscore in this Group was 41.9 ± 11 which was significantly higher compared to the non laparotomoy group (10,5 ± 1, $p<0.001$). However, this paper does not aim at the incidence or clinical causes and risk factors of mesenteric ischemia, but at identifying and validating early markers. Therefore, the focus of the analysis was on the markers and not the clinical data.

Question#c: Could you provide any comparisons between patients with NOMI or non-NOMI?

Answer#c: Since we have not proven an occlusive disease in any of the patients, this comparison is unfortunately not possible.

Question#d: How did you treat the patients with MESI?

Answer#d: In addition to the attempt to reduce the necessary catecholamine doses and generate an optimal cardiac output, the treatment focused primarily on the resection of the necrotic intestinal segment. At the time of the study, no attempts were made by vasodilators to improve mesenteric perfusion locally or systemically.

Question#e: Could you provide a table with the type of operations in patients with laparotomy?

Answer#e: As already described above, the laparotomized patients are seriously ill patients who have entered a postoperative cycle of reduced intestinal perfusion and the resulting reduced heart and lung performance. The intitial operation plays only a minor role here. In ouf opinion a table of operations would not add any deeper insight about the markers. The focus of this work is on markers and their course, as well as the observation that they can predict very early a mesenteric low perfusion, which ends in a laparotomy.

3. Discussion:

Question#a: Are these outcomes going to change sth in your daily practice?

Answer#a: If affordable I would use these markers as very early markers for general hypoperfusion with effect to the mesenterium. In my daily practice today I try to avoid catecholamines as much as possible and early on I set the indication to additional extracorporeal support systems to ensure the best possible perfusion.

Question#b: Why should you perform a laparotomy and not a laparoscopy first?

Answer#b: In fact, a laparotomy to exclude intestinal ischemia would be much more gentle, faster and in doubt even bed-side feasible. During the study period, however, our colleagues in general surgery performed only a few laparoscopic procedures and the argument of not being able to perform resections was put forward. Therefore, all laparotomies were performed openly. Meanwhile, laparoscopies are also performed.

4. General comments:

Question#A: The topic of your research is very important and interesting. However, the presentation of the cohort is a little bit difficult. You have to explain the type of MESI; if all cases were NOMI, then you have to change the terminology.

Answer#A: As already described in the introduction, the cause of mesenteric ischemia with the final picture of mesenteric necrosis can be manifold. The term NOMI merely describes a possible etiology of mesenteric ischemia. Further causes may be embolic events and thus occlusive mesenteric ischemia, reduced cardiac output or venous congestion. In cardiosurgical patients the events will mostly be multimodal, therefore the classification NOMI and OMI was deliberately avoided but used for a clear classification of the groups MESI in the sense of a safe clinically and pathologically proven mesenteric ischemia.

Question#B: Try to determine if there was a correlation between type of operation, op time and ICU stay with the need of laparatomy or MESI. By this way, you can recommend that in these types of patients (e.g. patient after mitral valve replacement with op duration > 4h, and ICU stay > 2 days) you have to check these markes. This would be very helpful for the readers.

Answer#B: No differences were found between the groups with and without laparotomy with respect to mean age ($p=0.182$), sex ($p=0.443$), BMI ($p=0.234$), left ventricular function (0.817), preoperative NYHA classification ($p=0.367$), hypertension ($p=0.072$) or coronary artery disease ($p=0.281$). Patients with laparotomy during the postoperative course were found to have significantly more cardiovascular risk factors with a higher rate of diabetes (61% vs 27%, $p=0.002$), more smoking (50% vs 18%, $p<0.001$) and more peripheral artery disease (61% vs 15%, $p<0.001$). Furthermore patients with laparotomy during the postoperative course had longer bypass times (151 ± 74 min vs. 120 ± 55 min $p=0.023$) with CPB time > 150 min in 67% vs 21% of the patients ($p<0.001$). The rate of patients with need for inotropic support already before cardiac surgery was higher in the laparotomy group (38.9% vs. 3.5%, $p<0.001$). Also, postoperatively atrial fibrillation (12% vs 28%, $p=0.043$) and heart failure (8.6% vs 39%, $p<0.001$) were more frequent in patients requiring laparotomy later. Laparotomy patients demonstrated significantly higher GICS scores (12.97 vs. 3.13, $p<0.001$) and Euroscore II scores (30.75 vs. 6.6, $p<0.001$). Some of these parameters were included into the results section and mentioned later in the discussion. We could now easily calculate uni- and multivariate models and quantify these correlations and call them risk factors. Nevertheless, these results about clinical risk factors repeat what we already know and what has been published before. Our focus was to study new markers, not known risk factors.

Question#C: You are talking about early diagnosis but it seems that this will not influence the outcome according to the literature in case of NOMI. Did I understand it right? In this case, you have to discuss the real benefit of early detection.

Answer#C: We completely agree. On the other side there is not only black and white. In our understanding and from our experience in rat models we know, that MESI is a dynamic process involving other organs. It seems like any use of CPB or hypotension, even running a marathon, leeds to some degree of mesenteric malperfusion. Depending on the duration and the recovery capacity different degrees of damage occure. We were looking for markers that could help us to get a deeper understanding of these processes after heart surgery. Therefore, we wrote in the discussion section: “The definition of MESI as intestinal ischemia treated by laparotomy and resection is unambiguous and used in most retrospective studies. However, it does not reflect the dynamics of this disease and its pathomechanism which is non-occlusive (NOMI) in 95% [3] with an incidence after cardiac surgery up to 10% [23]. We assume an even higher rate of mild and non-apparent MESI after most cardiac operations demonstrated by elevated markers after CPB in all studies including ours. Although αGST and D-lactate seem to be suitable markers for early detection of gastrointestinal complications after cardiac surgery and iFABP-2 could help differenciating patients with ischemia, resection of necrotic tissue is only damage control, and the benefit of a very early resection is questionable. Accepting the pathologic mechanism and the dynamic character new therapeutic and protective approaches are required. Substances like Glycine [24,25] and Pyruvate [26] demonstrated protective effects in MESI models in rats. The evaluated markers could not only be useful for early detection of patients with mesenteric ischemia, but also represent a new routine for evaluation of protective effects of different substances or conditioning maneuvers in further clinical studies.”

Reviewer#2

Question#1: Do you think that the retrospective measurement of all markers in the blood samples could bias the results? Where and how did you preserve the samples?

Answer#1: In fact, the retrospective measurement of the samples could change the actual value. The samples were stored at -80°C after centrifugation and collected the regular laboratory parameters and then measured in larger groups to have sufficient samples for the rather expensive essays. In preliminary experiments with animal samples, the duration of the sample storage had no influence on the measurement result. If, however, a non-excludable error should have occurred due to the time interval until storage, this would be a systematic error that should not have led to any bias.

Question#1: There was a similar study published by Ludewig et al. in 2017. Why was this study not cited or the results discussed since they are completely different to the results of the presented study?

Answer#1: This manuskript was mainly written with one interruption for an international fellowship before the Ludewig paper was published, therefore it was not recognized and cited. However, the authors explain thereselves: "It may be possible that I-FABP is not released when the ischemia of the bowel wall progresses and the mucosa does not recover, leading to false negative I-FABP test results". In our study only patients with proven bowel necrosis were categorized as MESI patients and therefore they might have no more I-FABP to release as stated in our discussion. On the other hand nearly our complete control group underwent cardiopulmonary bypass before the first postoperative measurement. Based on the high sensitivity of the marker we can expect elevated markers in nearly all patients, even in the control group, except those that have really severe damage, that does only recover within the next days.

Question#2: How was the decision for performing a laparotomy made? CT-based? Clinical state?

Answer#2: In 7/18 patients a CT and one angiography was performed before laparotomy, without any evidence of occlusion of an intestinal vessel. In most cases, however, the intestinal vessels were narrow and reduced in perfusion. However, since the CT findings were rarely unambiguous, laparotomy was performed in conjunction with the clinical signs. (We added the sentences: "Indication for laparotomy was primarily given by clinical signs for an acute abdomen, continuous hyperlactatemia or increasing need for vasopressors. Additional CT-imaging without evidence for any occlusive mesenteric malperfusion was performed in 7/18 patients. Angiography was performed in one patient")

Question#3: The number of patients is too low to draw a significant conclusion. Only 1,5 % of the observed patients received a laparotomy, the study design seems to be a random shot.

Answer#3: The study cohort includes 567 patients. Serum samples 1, 24 and 48h after cardiac surgery of 510 (90%), 502 (89%), and 313 (55%) patients respectively and 18 patients up to 72h before laparotomy were analysed. Basically we compared the markers of 18 patients with laparotomy with 510 patients without. The group of laparotomy patients (3.6%) looks small but the effect must have been strong enough, so that the differences were statistically significant. The study presented here was planned following a pilot study. As part of the pilot study, we collected blood samples from patients suspected of having mesenteric ischemia who were laparotomized shortly afterwards. To our surprise, the measured values at this time shortly before the laparotomy were significantly lower than expected. On the other hand, the values measured early postoperatively as a control group were higher. We concluded from this that probably every use of the HLM causes a mesenteric ischemia of small magnitude and that the release of markers is no longer to be expected if the tissue is already in a position to measure as many meaningful samples as possible with the limited financial resources, we decided on the selected study design, in which we first observe the early postoperative markers and the early postoperative course of all patients and in patients who undergo a laparotomy also the period before the laparotomy. This procedure seemed to us to be reasonable and was no coincidence. Retrospectively, we should also have taken preoperative samples, but we could not do this after the study.

Question#4: IFABP is mainly produced in the small intestine, but only two patients had a small bowel ischemia. How do the authors interpret this?

Answer#4: We completely agree with you, I-FABP is expressed in a significantly higher proportion in the small intestine. Nevertheless, an expression in the large intestine is also described. Furthermore, the fact that only in 2 patients a resection worthy necrosis of the small intestine was found does not mean that in the remaining patients with a resection of the large intestine not also an intraluminal but not a transmural damage of the small intestine is present.

Question#5: How many patients needed a perioperative renal replacement therapy?

Answer#5: Out of the total cohort 82.5% needed no postoperative renal replacement therapy, 15% received temporary postoperative renal replacement therapy, 2.5% needed intermittend dialysis postoperatively. Temporary postoperative dialysis therapy was significantly more frequent in patients that needed a laparotomy during the postoperative course (33% vs 14.4% $p<0.001$). We do not know how many of the patients had intraoperative dialysis, but agree that CVVHD intra- and postoperatively, as well as the renal function might have influenced the FABP values. We were unfortunately unable to exclude this variable.

Question#6: How many patients suffered from liver cirrhosis?

Answer#6: We did not store any information about the liver function, but agree, that it might have influenced the markers as well.

Reviewer#3

Question#1: I would be interested in the number of patients in your study who died because of MI after cardiac surgery and were not presented

for surgery?
Answer#1: All patients suspicious for mesenteric ischemia underwent laparotomy.

Reviewers' Comments to Revision

Reviewer 1: Theodosios Bisdas

Nov 05, 2018

Reviewer Recommendation Term:	Accept
Overall Reviewer Manuscript Rating:	80

Custom Review Questions	**Response**
Is the subject area appropriate for you?	4
Does the title clearly reflect the paper's content?	4
Does the abstract clearly reflect the paper's content?	4
Do the keywords clearly reflect the paper's content?	4
Does the introduction present the problem clearly?	4
Are the results/conclusions justified?	4
How comprehensive and up-to-date is the subject matter presented?	4
How adequate is the data presentation?	4
Are units and terminology used correctly?	4
Is the number of cases adequate?	4
Are the experimental methods/clinical studies adequate?	4
Is the length appropriate in relation to the content?	3
Does the reader get new insights from the article?	3
Please rate the practical significance.	3
Please rate the accuracy of methods.	3
Please rate the statistical evaluation and quality control.	5 - High/Yes
Please rate the appropriateness of the figures and tables.	4
Please rate the appropriateness of the references.	4
Please evaluate the writing style and use of language.	4
Please judge the overall scientific quality of the manuscript.	4
Are you willing to review the revision of this manuscript?	Yes

Comments to Authors:
I have no further comments.

Reviewer 2: anonymous

Nov 05, 2018

Reviewer Recommendation Term:	Reject
Overall Reviewer Manuscript Rating:	25

Custom Review Questions	**Response**
Is the subject area appropriate for you?	4
Does the title clearly reflect the paper's content?	4
Does the abstract clearly reflect the paper's content?	3
Do the keywords clearly reflect the paper's content?	3
Does the introduction present the problem clearly?	3
Are the results/conclusions justified?	3

How comprehensive and up-to-date is the subject matter presented?	3
How adequate is the data presentation?	3
Are units and terminology used correctly?	3
Is the number of cases adequate?	1 - Low/No
Are the experimental methods/clinical studies adequate?	3
Is the length appropriate in relation to the content?	3
Does the reader get new insights from the article?	2
Please rate the practical significance.	2
Please rate the accuracy of methods.	3
Please rate the statistical evaluation and quality control.	3
Please rate the appropriateness of the figures and tables.	3
Please rate the appropriateness of the references.	1 - Low/No
Please evaluate the writing style and use of language.	3
Please judge the overall scientific quality of the manuscript.	3
Are you willing to review the revision of this manuscript?	Yes

Comments to Authors:
Thank you for the revised version of the manuscript.
There are still major flaws which do not convince me that the paper should be published in its present form.
1. The paper of Ludewig et al. is important in this context. Even if the authors have written their paper in 2017, it is submitted in 2018! Therefore, it should be possible to include a one year old citation!
2. I am convinced that the renal replacement therapy influences the results. Therefore, it is not conclusive that the significant values of the small cohort of the mesenteric ischemia patients are only an effect of the ischemia and might be furthermore influenced by the renal dysfunction as well.
3. It should be possible to include data about the liver function in a revised manuscript! The case series is retrospective and I am convinced that data of liver function are available.

Reviewer 3: anonymous

Nov 05, 2018

Reviewer Recommendation Term:	Accept
Overall Reviewer Manuscript Rating:	50

Custom Review Questions	**Response**
Is the subject area appropriate for you?	3
Does the title clearly reflect the paper's content?	4
Does the abstract clearly reflect the paper's content?	4
Do the keywords clearly reflect the paper's content?	4
Does the introduction present the problem clearly?	4
Are the results/conclusions justified?	3
How comprehensive and up-to-date is the subject matter presented?	4
How adequate is the data presentation?	3
Are units and terminology used correctly?	4
Is the number of cases adequate?	3
Are the experimental methods/clinical studies adequate?	3
Is the length appropriate in relation to the content?	4
Does the reader get new insights from the article?	3
Please rate the practical significance.	3
Please rate the accuracy of methods.	3
Please rate the statistical evaluation and quality control.	3
Please rate the appropriateness of the figures and tables.	4

Please rate the appropriateness of the references.	4
Please evaluate the writing style and use of language.	4
Please judge the overall scientific quality of the manuscript.	3
Are you willing to review the revision of this manuscript?	Yes

Comments to Authors:

This is a nice paper which describes the difficulty of discover a mesenteric ischemia after cardiac surgery. There are no standard clinical markers after surgery. Normally it is a combination of clinical examination an high serum lactat. But lactat after cardiac surgery is often high because of the low cardiac output after cardiac surgery in patients with preoperativ low ejection fraction (EF). This results in a relative ischemia with higher serum lactat. Most mesenteriac ischemia are non occlusive so diagnostic with CT scan shows the extent of mesenteriac ischemia just in an advaced stage, when a surgery is almost to late. Non occlusive MI is often a result of high catecholamines in patients with low cardiac Output, so it is a circulus vitiosus. The idea of finding markers for MESI is really important and make the decision for a laparotomy in patients after cardiac surgery easier.

Authors' Response to Reviewer Comments

Nov 12, 2018

Many thanks for the opportunity for an academic discussion. Please find below the answers to the questions of reviewer 2:

Reviewer 2, comments to the revised manuscript:

Question 1:

The paper of Ludewig et al. is important in this context. Even if the authors have written their paper in 2017, it is submitted in 2018! Therefore, it should be possible to include a one year old citation!

Answer 1:

The interest of the independent reviewer in this specific paper and its citation is interesting. As already explained in the review, the trivial reason not to cite this paper was the time of writing. In addition, however, we also explained why Ludewig et al. were not cited, but without going too deeply into the paper in question. I would like to make up for that.

Ludewig et al., similar to our previously mentioned pilot study, have included patients with suspected intestinal ischemia. In an interdisciplinary intensive care unit with over 2000 patients in the study period, a total of 43 patients with suspected ischaemia of the intestine were included. Of these, 21 patients were laparotomized, intestinal ischemia / necrosis was confirmed in 20 patients and parts of the bowel were resected. In 22 patients the survival of 7 days was interpreted as the exclusion of intestinal ischemia.

We consider the exclusion of intestinal ischemia by survival of 7 days without laparotomy to be a very questionable endpoint. As von Schellekens et al (2014) shows, in a human model the iFABP concentration in the serum decreased already after 120 min of ischemia by destruction of all enterocytes and villous structures. As the authors themselves proved (1 patient was not resected but only embolectomized) such an ischemia does not necessarily lead to resection and a missing laparotomy does not rule out intestinal ischemia. The question is therefore more about the definition of intestinal ischemia. Do we define it as a necrotic intestinal segment that has to be resected or as the destruction of all enterocytes and villous structures, and how we describe the stages in between? Therefore, we have consistently chosen the endpoint laparotomy. In addition, we chose another reference point for the measurement: the laparotomy itself, which was performed on average 11 days after the initial heart operation.

The patient population of the two studies is therefore difficult to compare. In the group of patients with intestinal ischemia at Ludewig et al. only 19% of the patients were operated with CPB, in group 2 only 36%. The collective chosen by the colleagues is more heterogeneous, as probably also the etiology of mesenteric ischemia. While in our work heart surgery was chosen as the presumed trigger, in Ludewig et al. the first sample was obtaineda at the time of suspicion of intestinal ischemia, or the sample obtained the day before was used. Due to the different reference points in our study design, different time periods of a dynamic event are considered. In our work, however, our study design clearly defined the reference points with a) the heart operation as trigger and b) the laparotomy.

In the overall view, however, the results are not necessarily contradictory. Also Ludewig et al only found a significant difference between the groups with and without mesenteric ischemia in serum samples in the subgroup analysis with only 22 patients, whose presumed triggering event was less than 48 hours ago. Thus, as our data with two further markers show, within the first hours after the trigger event, the markers seem to be increased or to rise. In the case of iFABP, which probably rises intraoperatively already very quickly after damage. It is not so surprising that after longer surgeries the markers are already lowered directly postoperatively, especially in comparison to a collective that also

had an operation with CPB and whose postoperative values are all raised compared to a healthy non-operated collective (in the context of the unpublished pilot study collected data). The differences between laparotomised patients with and without intestinal ischemia in which the patients with mesenteric ischemia showed significantly lower iFBAP values can also be reconciled with the Ludewig et al study. In their study already after 48h 1/3 of the tests were “false negative”, after 96h all tests were “false negative”. In our collective, the lapartomy was performed on average 11 days after the triggering event.

To include the work of Ludewig et al. in the discussion of our manuscript would become a very complex discourse on the different study designs, different collectives and different measurement methods. The ductus of the argumentation used here has already been used in the same way in the discussion of our manuscript. Therefore, we believe that the additional discussion of Ludewig et al. does not offer any new aspects, which is why we prefer not to cite it.

Question 2:

I am convinced that the renal replacement therapy influences the results. Therefore, it is not conclusive that the significant values of the small cohort of the mesenteric ischemia patients are only an effect of the ischemia and might be furthermore influenced by the renal dysfunction as well.

Answer 2:

Since FABP is eliminated renal, I agree with your idea. However, even after re-analysis of patients with laparotomy as well as patients with mesenteric ischemia, we could not detect any significant difference in the iFABP plasma concentrations of patients with and without CVVHD at -72h (p=0.85), -48h (p=0.36), -24h (p=0.92), p=-12h (0.52) before laparotomy. Perhaps we could have found an effect with a larger collective.

Question 3:

It should be possible to include data about the liver function in a revised manuscript! The case series is retrospective and I am convinced that data of liver function are available.

Answer 3:

I agree with the reviewer that data on liver function exist in the patients charts and that these could be added to our existing database. However, there are two major problems. On the one hand, the re-selection of the “liver parameters” (I assume the reviewer means transaminases and INR / albumin as synthesis parameters) would mean an additional logistic effort which is unrealistically high, especially since I am no longer working in the clinic where the study was conducted. On the other hand, the endpoints are chosen very consciously. In a further study on organ damage parameters in which we also examined the “liver parameters” we saw a 36h delayed increase of the “liver parameters” in every patient after HLM. In the already mentioned pilot study (which admittedly included fewer patients) we could not find any relevant influence, if at all a connection with aGST could be established. Certainly liver function will also have an influence on aGST release and D-lactate degradation, as well as on mesenteric perfusion itself, and on cardiac function. To fully uncover these relations is a very large project on which we have continued to work with animal experiments, but whose translation into clinical practice is very complex and was not the aim of the submitted publication, nor could have been.