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Plasma D-Lactate as a Potential Early Marker for Colon Ischaemia After Open Aortic Reconstruction

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Background and aim. *The breakdown of mucosal barrier function due to intestinal hypo-perfusion is the earliest dysfunction of ischaemic colitis. Severe colon ischaemia after aortic reconstruction is associated with mortality rates up to 90%. Therefore, early detection and treatment of patients with extensive ischaemic colitis is of crucial importance. In experimental studies, both* D*-lactate and bacterial endotoxin have been reported as markers of intestinal mucosal barrier impairment. However, evidence of their value in clinical practice is lacking. The aim of this pilot prospective cohort study was to assess the association between ischaemia of the colon (assessed histologically) and plasma levels of* D*-lactate and endotoxin in patients undergoing open aortic reconstruction.*
Patients and methods. *Twelve consecutive patients underwent surgery between February and April 2003. Six patients underwent emergency surgery and six patients elective aortic surgery.* D*-Lactate and endotoxin levels were measured in blood samples collected according to a standardised protocol. For histological examination biopsies were obtained by sigmoidoscopy on days 4–6 after surgery, or earlier if indicated clinically.*
Results. *As early as 2 h postoperatively, elevated plasma levels of* D*-lactate were measured in patients with histologically proven ischaemic colitis. The peak of* D*-lactate elevation was on postoperative days 1 and 2. Concentration of plasma endotoxin was not significantly different in patients with or without ischaemic colitis.*
Conclusion. *Our data suggest that plasma* D*-lactate levels are a useful marker for early detection of ischaemic colitis secondary to aortic surgery.*

Keywords: Aortic surgery; D*-lactate; Colon ischaemia; Ischaemic colitis; Sepsis.*

Introduction

The reported incidence of colon ischaemia after open aortic surgery for infrarenal aortic aneurysm varies between 3% for elective surgery and 36% for emergency surgery[1–4] with mortality rates of up to 90%.[5] Many factors have been associated with asymptomatic and symptomatic colon ischaemia, which is predominantly affecting the sigmoid colon. Causes for the development of colon ischaemia include hypo-perfusion, embolisation and reperfusion injuries.[6–9] Early detection of this condition is essential to ensure the rapid, correct treatment. Yet, laboratory parameters with acceptable sensitivity and specificity, or other tools for early detection, have not proven helpful in diagnosing intestinal ischaemia in routine clinical practice.

Based on the concept that breakdown of mucosal barrier function due to intestinal hypo-perfusion is the earliest dysfunction of ischaemic colitis, detection of translocation parameters might allow prompt diagnosis of early tissue necrosis.[10–14] Two promising analytes are D-lactate and endotoxins (lipopolysaccharides = LPS), which are metabolic products or components of the normal anaerobic bacteria of the gastrointestinal tract. Plasma D-lactate levels have been reported to be increased early after the damage to the intestinal mucosal barrier in animal models.[15,16] Plasma D-lactate was also elevated in patients with colon ischaemia in a retrospective study.[17] LPS, major

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constituents of the outer membrane of Gram-negative bacteria, are released from bacteria when replicating, dying or lysing.[18,19] Increased LPS levels have been related to impaired mucosal barrier.[20] However, contradictory evidence concerning the association between circulating endotoxin levels and ischemia induced intestinal injury has been reported.[21,22]

The aim of this prospective pilot cohort study was to assess the clinical significance of D-lactate and LPS as early markers for ischaemic colitis, proven by histology.

Patients and Methods

Patients

Twelve consecutive patients underwent aortic surgery between February and April 2003. Six patients needed emergency surgery (five patients with ruptured, and one patient with symptomatic infra-renal aortic aneurysm larger than 5.5 cm in diameter) and six patients underwent elective aortic surgery (two patients with an asymptomatic infra-renal aortic aneurysm larger than 5.5 cm in diameter, one patient with a chronic aortic dissection, and three patients with infra-renal aortic occlusion). Eleven patients were male, one female, the age ranged from 48 to 82 years (median = 62 years; IQR 61–75). All patients gave written informed consent. The study was conducted in adherence to the declaration of Helsinki.

Blood samples

Blood was drawn preoperatively from a central venous catheter in all patients undergoing elective surgery. In two patients undergoing emergency surgery, a preoperative sample was collected; in four patients the circumstances precluded collection of a preoperative blood sample.

Blood was collected according to a standardised protocol preoperatively (at day of surgery, sample A), 2 h after skin closure (sample B), 24 h (sample C), and 48 h (sample D) thereafter. Briefly, whole blood (8 ml) was collected in an EDTA tube and 5 ml in a heparinized endotoxin free tube (Endo Tube ET, Chromogenix, Austria). After the blood was drawn, it was immediately centrifuged at 2000*g* for 10 min and stored at −20 °C until further analysis.

Plasma D-lactate measurement

Plasma D-lactate levels were measured by a slight modification of the method described earlier.[23] Briefly, heparin plasma was deproteinised with 4 M perchloric acid and neutralized with 2 M K_2CO_3. D-lactate concentrations were determined by coupled enzymatic reactions utilizing D-lactate dehydrogenase and alanine aminotransferase. The formation of NADH at pH 8.0 was measured at 340 nm.

Plasma endotoxin measurement

The plasma endotoxin concentration was measured by the limulus amebocyte lysate test with a kinetic procedure, using the Endochrome K test kit (Coa-Chrom, Vienna, Austria). Briefly, heparinised plasma was diluted 1:10 in pyrogen free water and incubated at 75 °C for 10 min to remove non-specific inhibitors. The detection limit of this method is 0.75 pg/ml.

Histological evaluation

Sigmoidoscopy with biopsies was performed in all patients on days 4–6 after surgery or when earlier if clinically indicated. A single dose of 2 g cefuroxime i.v. was given prior to the procedure. Biopsies were taken at 20, 30 and 40 cm from the anal verge. All specimens were immediately stored in 5% formaldehyde solution for histopathological examination. Visible mucosal abnormalities were also biopsied in addition to the standard specimens. Patients who did not survive beyond day 5 after surgery underwent autopsy; specimens of all organs were examined histologically.

Statistical analysis

Analysis of the data was performed using Epi Info v1.1.2 (CDC, Atlanta, GA). To assess the statistical strength and differences of D-lactate levels in patients with or without histologically proven colon ischaemia, means and standard deviation of continuous variables were plotted. Based on the null hypothesis of no differences in the means of the two patient-groups, *p*-values were calculated using a two tailed Student's *T*-test. For all tests, a *p*-value <0.05 was considered statistically significant.

Results

Patient characteristics are shown in Table 1. Histological examinations during the observation period of

Table 1. Patient characteristics

Patient	Sex	Age	Diagnosis	Ischemic colitis	Outcome
#1	M	82	RAAA	Yes	Died day 3
#2	M	60	RAAA	Yes	Died day 3
#3	M	74	RAAA	Yes	Died day 31
#4	M	77	RAAA	No	Discharged home
#5	M	77	RAAA	Yes	Discharged home
#6	M	61	SAAA	No	Discharged home
#7	M	61	EAAA	No	Discharged home
#8	F	62	EAAA	No	Discharged home
#9	M	63	AOD	No	Discharged home
#10	M	62	AOD	No	Discharged home
#11	M	51	AOD	No	Discharged home
#12	M	48	Dissection	No	Discharged home

RAAA, ruptured abdominal aortic aneurysm; SAAA, symptomatic abdominal aortic.

Table 2. D-Lactate levels

Patient	Sample A (μg/ml)	Sample B (μg/ml)	Sample C (μg/ml)	Sample D (μg/ml)
#1	1.40	3.31	3.74	3.46
#2	NA	1.95	3.01	4.51
#3	NA	2.00	4.60	1.55
#4	NA	2.06	1.98	1.83
#5	1.40	0.47	0.75	1.55
#6	NA	0.70	1.97	1.91
#7	0.55	1.08	NA	0.76
#8	0.78	0.49	0.34	1.53
#9	1.06	0.37	1.13	0.87
#10	1.01	0.43	1.55	1.14
#11	1.68	0.72	0.95	1.28
#12	1.26	0.84	0.84	NA

Sample A, preoperatively; sample B, 2 h after skin closure; sample C, 24 h postoperatively; sample D, 48 h postoperatively; NA, no blood samples drawn.

7 days revealed ischaemic colitis in four patients (patients #1, 2, 3, 5). D-lactate dynamics are shown in Fig. 1 and Table 2. Patients with histologically proven ischaemic colitis demonstrated significantly elevated D-lactate levels as early as 2 h after surgery and levels tended to remain high for the next 2 days.

Patient #1 had D-lactate elevation as early as 2 h postoperatively, with continued high lactate levels on days 1 and 2. He was stable until day 2 with one episode of faecal occult blood positive diarrhoea and deteriorated rapidly thereafter. The patient died on day 3 and autopsy revealed colon gangrene, predominantly of the sigmoid colon.

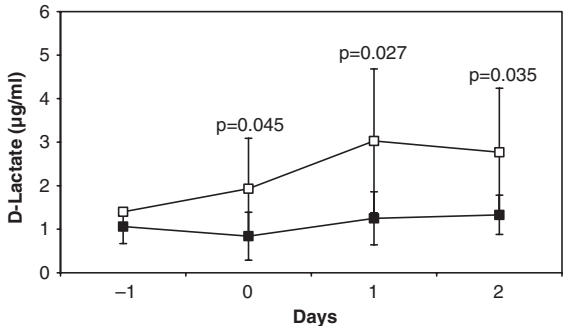

Fig. 1. Plasma D-lactate concentrations in patients undergoing aortic surgery. Open squares represent D-lactate concentrations in patients developing histologically proven ischemic colitis; filled squares represent D-lactate concentrations in patients without ischemic colitis. Data are presented as mean±SD. P values represent significance level *versus* corresponding group without ischaemic colitis.

Patient #2 was stable after surgery and on dialysis. He deteriorated on day 2 without any indication of the cause of clinical decline. Autopsy revealed colon and intestinal necrosis. Again, D-lactate rise preceded clinical decline.

Patient #3 had the highest D-lactate level 24 h postoperatively. However, sigmoidoscopy was normal without histological changes. This patient had a tender abdomen on day 3, after a myocardial infarction. CT of the abdomen revealed coecal necrosis and a right hemicolectomy was performed on day 7. He ultimately died of multiple myocardial infarcts and renal insufficiency on day 31.

Patient #5 had no clinical signs or symptoms of ischaemic colitis. Colonoscopy on day 5 revealed a patchy mucosa with histological changes consistent with ischaemic colitis. The patient was discharged home and had a histologically normal mucosa 1 month postoperatively. In contrast, LPS levels were not changed prior during or after clinical decline in these patients.

Mean plasma D-lactate levels of patients with ischaemic colitis were 1.40 ± 0.00, 1.93 ± 1.16, 3.03 ± 1.65, and 2.77 ± 1.47 μg/ml; in patients without ischaemic colitis 1.06 ± 0.39, 0.84 ± 0.55, 1.25 ± 0.61 and 1.33 ± 0.45 μg/ml, respectively. Plasma D-lactate levels of patients with ischaemic colitis compared to those without as demonstrated by histology were significantly higher on days 0, 1 and 2 ($p=0.045$; $p=0.027$ and $p=0.035$, respectively).

The time course of plasma endotoxin levels are shown in Table 3. No association with ischaemic colitis, proven by histological examination, could be demonstrated.

Table 3. Endotoxin (LPS) levels

Patient	Sample A (EU/ml)	Sample B (EU/ml)	Sample C (EU/ml)	Sample D (EU/ml)
#1	<0.1	<0.1	<0.1	<0.1
#2	NA	0.15	<0.1	<0.1
#3	NA	<0.1	0.12	<0.1
#4	NA	<0.1	<0.1	<0.1
#5	<0.1	<0.1	<0.1	0.14
#6	NA	<0.1	<0.1	0.15
#7	<0.1	<0.1	<0.1	<0.1
#8	0.15	<0.1	0.31	<0.1
#9	<0.1	0.18	0.60	<0.1
#10	<0.1	0.2	<0.1	<0.1
#11	<0.1	0.2	<0.1	<0.1
#12	0.14	<0.1	<0.1	NA

Sample A, preoperatively; sample B, 2 h after skin closure; sample C, 24 h postoperatively; sample D, 48 h postoperatively; NA, no blood samples drawn.

Discussion

The intestinal mucosa is highly sensitive to ischaemic insults. A breakdown of the mucosal barrier is supposed to be an initiating event in the development of sepsis and MOF.[14,24,25] The underlying cause for the development of colon ischaemia after open aortic surgery may include the interruption of the inferior mesenteric artery and hypo-perfusion of the intestine from the hypogastric arteries during cross-clamping of the aorta.[22,25–28] In addition, the abdominal compartment syndrome, macro-, and micro-embolisation have been implicated.[8,9] The relative advantages of retroperitoneal *versus* trans-peritoneal approach also have been discussed.[29–31] A reliable blood marker is required for the early diagnosis of acute intestinal insult in clinical practice.

D-Lactate is produced by the normal anaerobic bacteria of the gastrointestinal tract.[32,33] In contrast to common belief, humans metabolise D-lactate at considerable rates with infused D-lactate having a half life of 20–40 min.[34] However, a leaky mucosal barrier may cause permanent translocation of D-lactate and the long half life, compared to that of LPS, could allow for accumulation. Therefore, D-lactate could be a useful clinical marker.

Our study indicates an association between plasma D-lactate levels and ischaemic colitis, early after surgery. We observed elevated levels of D-Lactate in patients with ischaemic colitis as early as 2 h postoperatively. The peak of D-lactate elevation was on days 1 and 2 postoperatively and preceded the clinical deterioration of patients, making this parameter possibly useful for early detection and initiation of subsequent therapy of ischaemic colitis secondary to aortic surgery.

LPS also has been reported to be an early marker of impaired mucosal barrier function after intestinal ischaemia/reperfusion injuries. It has been shown, that a correlation between serum D-lactate and LPS does exist in an experimental study.[20] However, in other studies no elevated LPS levels were measured after severe trauma, shock[21] or occlusion of the mesenteric artery.[22] The absence of an increase in plasma endotoxin levels in clinical settings may be due to the fast clearance and the short half-life of LPS in man. In our study, LPS did not appear to be a useful parameter, since no correlation between LPS, D-lactate and the clinical or histological findings could be demonstrated.

In conclusion, our data suggest a significant association between plasma D-lactate levels and histologically proven ischaemic colitis after aortic surgery. Therefore, D-lactate could be a useful guide to impaired intestinal mucosal barrier function in routine clinical practice. Larger prospective studies are warranted to establish a threshold for elevated D-lactate levels in patients after aortic surgery to confirm and extend our findings.

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