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Exploring D-Lactate as a Biomarker for Acute Intestinal Necrosis in 2958 Patients: A Prospective Cross-Sectional Study

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□ **Abstract—Background: Timely diagnosis of acute intestinal necrosis (AIN) is lifesaving, but challenging due to unclear clinical presentation. D-lactate has been proposed as an AIN biomarker. Objectives: We aimed to test the diagnostic performance in a clinical setting. Methods: We performed a cross-sectional prospective study, including all adult patients with acute referral to a single tertiary gastrointestinal surgical department during 2015–2016 and supplemented by enrollment of high-risk in-hospital patients suspected of having AIN during 2016–2019. AIN was verified intraoperatively, and D-lactate was analyzed using an automatic spectrophotometric set-up. A D-lactate cut-off for AIN was estimated using the receiver operating characteristic curve. The performance according to patient subgroups was estimated using the area under the receiver operating characteristic curve (AUC). Given the exploratory nature of this study, a formal power calculation was not feasible. Results: Forty-four AIN patients and 2914 controls were enrolled. The D-lactate cut-off was found to be 0.0925 mM. Due to lipemic interference, D-lactate could not be quantified in half of the patients, leaving 23 AIN patients and 1456 controls for analysis. The AUC for the diagnosis of AIN by D-lactate was 0.588 (95% confidence interval 0.475–0.712), with a sensitivity of 0.261 and specificity of 0.892. Analysis of high-risk patients showed similar results (AUC 0.579; 95% confidence interval 0.422–0.736). Conclusion: D-lactate showed low sensitivity for AIN in both average-risk and high-risk patients. Moreover, lipemic interference precluded valid spectrophotometric assessment of D-lactate in half of the patients, further disqualifying the clinical utility of D-lactate as a diagnostic marker for AIN. © 2024 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)**

□ **Keywords—mesenteric ischemia; lipemic interference; diagnosis; D-lactate**

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Trial registration: ClinicalTrials.gov. Nr.: NCT05665946, retrospectively registered. Link: Acute Intestinal Necrosis- the Preoperative Diagnostic Approach – Full Text View – ClinicalTrials.gov

Introduction

Acute intestinal ischemia and its end-stage acute intestinal necrosis (AIN) is a life-threatening disease with a high mortality rate (1). It is caused by one of three different mechanisms: 1) vascular or primary AIN caused by intravascular obstruction of the blood supply due to

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an embolus or a thrombus; 2) nonvascular or secondary AIN caused by external compression or strangulation of the intestinal vessels, such as herniation or adhesions; and 3) nonocclusive mesenteric ischemia caused by the collapse of the systemic or intestinal blood circulation. The resulting ischemia, if untreated, causes irreversible necrosis of the intestinal segment within approximately 6 h and eventually results in the death of the patient (2). In particular, vascular AIN has been associated with a high mortality rate of above 60% (1). Early diagnosis and intervention, therefore, are essential to ensure survival in AIN patients. The diagnosis is particularly challenging in vascular AIN in the early phase of the disease, where peritoneal signs and organ dysfunction are absent in most patients (3). Additionally, Lehtimäki et al. demonstrated in a retrospective study that AIN is underdiagnosed in abdominal computed tomography (CT) scans when clinical suspicion is absent (4). They showed that the abdominal CT scan has a sensitivity of 97% diagnosing AIN if the suspicion was mentioned in the CT referral, compared with a sensitivity of 81% if the suspicion for AIN was unmentioned. Altogether, the high mortality rate may be explained by late diagnosis violating the timeframe of the first 6 h (2), subsequently, delaying initiation of treatment due to an unclear clinical and paraclinical presentation at admission (5). The initial diagnostic process in acute patients, including AIN patients, relies mainly on clinical and paraclinical examinations, such as CT scans and standard blood-based parameters, and, ultimately, on surgery. Standard blood-based parameters include the widely used inflammation markers C-reactive protein and white blood cell count (4,6). Nevertheless, most standard blood-based parameters are unspecific. D-dimer attracts particular interest due to its negative predictive value of 1.00 with respect to AIN patients (7). L-lactate is unspecific as well, but is frequently used in spite of poor evidence (8). Reliable biomarkers to facilitate the early identification of patients suffering from AIN have been sought for decades without significant breakthroughs (9).

D-lactate has been suggested as a promising biomarker in a small number of animal and human studies (10–15). D-lactate is the stereoisomer of L-lactate, and contrary to L-lactate, the production of D-lactate is scarce in the human body and originates mainly from bacteria (*Lactobacilli, Bifidobacteria*) in the intestinal lumen (3,16). Additionally, small quantities of D-lactate originate from dietary intake through yogurt and sauerkraut (17). The D-lactate production is stimulated by growth of lactic acid bacteria, which is promoted in an acidic environment (17). Damage to the mucosal barrier is shown as early as 30 min after an AIN event, and autodigestion is damaging the intestinal wall, causing the translocation of D-lactate into the bloodstream (18,19). The extent of hepatic elimination has been unclear, but recently, the degrading potential of a specific protein, D-alpha carboxy acid dehydrogenase (D-LDH), has been clarified (17,20). D-LDH is located on the inner membrane of the mitochondria widely distributed in the human body, with highest concentration in the liver and the kidneys (17). The elimination by D-LDH is abundant, and approximately 50% of the elimination by L-lactate dehydrogenase (L-LDH) (17). Nevertheless, D-lactate has been demonstrated to be elevated in peripheral venous samples after an AIN event, suggesting D-lactate as a relevant biomarker for AIN (13,19).

Based on this knowledge, our primary aim was to evaluate D-lactate as a first-line biomarker for AIN in unselected patients acutely admitted with abdominal symptoms, and secondary, to evaluate D-lactate as a biomarker in hospitalized patients with a high clinical suspicion for AIN.

Methods

Setting and Study Population

This was a single-center, cross-sectional study. We prospectively and consecutively included all patients who were acutely referred to The Gastrointestinal Surgical Department at Aalborg University Hospital in Denmark from January 6, 2015 to June 9, 2016. In addition, we included in-hospital patients with a clinical suspicion for AIN (referred to as "high-risk patients") between June 10, 2016 and March 24, 2019. The inclusion period excluded several holidays due to the scarcity of staff required for blood sample processing (111 days in total). Thus, inclusion criteria for non-high-risk patients were patients referred to the Gastrointestinal Surgical Department. Inclusion criterion for high-risk in-hospital patients was a clinical suspicion for AIN. Exclusion criterion for both non-high-risk and high-risk patients was patients younger than 18 years. Aalborg University Hospital is a tertiary referral hospital with an uptake area of approximately 300,000 inhabitants for basic hospital functions and approximately 590,000 inhabitants for regional hospital functions in the northern part of Denmark.

Definition of AIN and Controls

The AIN diagnosis was confirmed after surgical verification of intestinal ischemia or intestinal necrosis in the small intestine or colon. Intestinal viability was determined visually by intestinal color and peristalsis (21–23). Intestinal ischemia was defined as recovery of the intestine during the operation. Intestinal necrosis was defined as macroscopically necrotic elements in the intestine requiring resection. Nonvascular AIN was diagnosed intraoperatively as a relevant nonvascular reason for intestinal

necrosis (e.g., adhesions, herniation, or volvulus). Vascular AIN was classified in patients without a clear reason for AIN and, often, an absence of pulsation or lack of blood flow in the intestinal arteries. All non-AIN patients were designated as control patients in both the high-risk and the non-high-risk patient groups. High-risk patients were defined as patients with abdominal pain and mono- or multiple organ failure demanding acute surgery.

Data Collection

The following information was registered at admission in the medical record: sex, weight, height, heart rate, blood pressure, temperature, tobacco use, current pregnancy, comorbidities (diabetes mellitus, hypertension, heart disease, inflammatory bowel disease, current cancer, gastrointestinal malignancy, and atherosclerosis), medicine use (including insulin use in diabetes and use of anticoagulation therapy), and previous surgery (year, hospital, and operational findings). The mortality date was extracted from the hospital database as well. Details of the data collected are described in Supplementary Materials 1 (available online).

Blood Sample Collection

Blood samples for D-lactate analysis were collected in 3-mL Vacuette® FC-mix tubes (Cat # 454513; Greiner Bio-One International GmbH), together with routine blood samples according to the protocol for blood sampling in the routine setting. The samples were drawn at admission after written and verbal consent were obtained according to the approval from the North Denmark Region Committee on Health Research Ethics. High-risk patients were included when AIN was suspected, and blood samples were collected in 3-mL Vacuette® FC-mix tubes after written and verbal consent were acquired and prior to surgery, in accordance with the above-mentioned approval.

Preanalytical Handling and Analysis of the Blood Samples

The blood samples were centrifuged at 2200 g at 20°C for 15 min. Plasma was stored at −80°C in a biobank until analysis of all samples at the same time. Preanalytical stability tests were designed to mimic actual routines of collecting and handling research project samples in a 24/7 setting at the acute emergency department of our hospital. Acknowledging the variation in workload, we designed a study to test the impact of worst-case scenarios regarding pre-analytical handling compared with the optimal workflow. Details of the stability test set-up are described in Supplementary Materials 2 (available online).

As the intention was to develop a 24/7 analytical set-up, the concentration of D-lactate in plasma was measured on our 24/7 automated chemistry equipment according to Rasmussen et al. (24). In brief, a D-lactate kit (K-DATE, Megazyme International Ireland, Co. Wicklow, Ireland) was applied in a modified set-up on a Roche Cobas c502 module (Roche Diagnostics A/S, Hvidovre, Denmark). To avoid interference from endogenous L-lactate and L-LDH, the fully automatic set-up included a step with a pH increase to inactivate L-LDH. Each reagent preparation was calibrated with the commercial kit calibrator and controlled with two repetitions of custom-made controls in four levels (0.05 to 1 mM) prior to and after each batch. The coefficient of variation was 9% (0.05 mM); the limit of blank was 0.02 mM, the limit of detection (LoD) was 0.04 mM, and the limit of quantification (LoQ) was 0.05 mM. No interference was observed from a hemolytic index up to 500 or icteric index up to 50. Significant interference was observed from lipemia. We defined acceptable interference by lipemia as 20% corresponding to a Lipemia index (L-index) of 60 (at D-lactate cut-off: 0.3 mM proposed by previous studies) (20). Applying 20% interference to the cut-off calculated from data in this study (D-lactate cut-off: 0.0925 mM) resulted in an adjustment of the L-index to 22 (20). The analytical process was blinded. Additionally, validation studies were performed according to state-of-the-art procedures and recently published in the study by Rasmussen et al. (24). Research is being reported in line with the Strengthening the Reporting of Cohort Studies in Surgery Guidelines (25).

Ethics

This study was approved by the North Denmark Region Committee on Health Research Ethics (N-20170089).

Statistical Analysis

The mean and standard deviation (SD) for D-lactate are reported corrected for the LoQ. D-lactate levels below the LoQ were given a value of 0.025 mM, representing 50% of the LoQ according to Antweiler and Taylor (26).

Additionally, the fraction of D-lactate measurements below the LoQ is reported. The D-lactate distribution is right skewed; however, as a large proportion is below the LoQ, medians and interquartile range may not be informative. Categorical variables are expressed as numbers and percentages. Continuous variables are reported as the mean and SD. The difference between AIN patients and controls in proportions of comorbidities is quantified by the risk difference and 95% confidence interval. These are calculated by generalized linear models with iden-

Table 1. Characteristics of 1479 Acute Surgical Patients with Lipemic Index < 22 with D-Lactate Estimation.

	Controls	AIN Patients		
Variable	n (%)	n (%)	Risk Difference % (95% CI)	*p* Value
Patients	1456 (100.0)	23 (100.0)		
Male	659 (45.3)	9 (39.1)	−6.1 (−26.2–14.0)	0.550
Age above 70 years	493 (33.9)	16 (69.6)	35.7 (16.7–54.7)	0.000
Tree month mortality	113 (7.8)	7 (30.4)	22.7 (3.8–41.5)	0.018
Age, years: mean (SD)	57.0 (21.2)	75.4 (10.5)	18.4 (14.0–22.7)	0.000
Diabetes mellitus	152 (10.4)	3 (13.0)	2.6 (−11.3–16.5)	0.713
Insulin-dependent DM	51 (3.5)	0 (0.0)	−3.5 (−4.4 to −2.6)	0.000
Hypertension	446 (30.6)	13 (56.5)	25.9 (5.5–46.3)	0.013
Heart disease	303 (20.8)	8 (34.8)	14.0 (−5.6–33.6)	0.162
Arteriosclerotic disease	312 (21.4)	10 (43.5)	22.0 (1.7–42.4)	0.034
Cancer	255 (17.5)	2 (8.7)	−8.8 (−20.5–2.9)	0.139
GI cancer	29 (2.0)	0 (0.0)	−2.0 (−2.7 to −1.3)	0.000
Former abdominal operations	689 (47.3)	11 (47.8)	0.5 (−20.1–21.1)	0.962
Active smoking	325 (22.3)	5 (21.7)	−0.6 (−17.6–16.4)	0.946
Use of medication	1100 (75.5)	23 (100.0)	24.5 (22.2–26.7)	0.000
Use of anticoagulants	406 (27.9)	15 (65.2)	37.3 (17.7–56.9)	0.000

Difference for categorical variables is difference in proportion (risk difference) and for continuous variables difference in mean.
AIN = acute intestinal necrosis; CI = confidence interval; SD = standard deviation; DM = diabetes mellitus; GI = gastrointestinal.

tity links and robust calculations of standard error. The diagnostic performance of D-lactate according to AIN status was assessed by the receiver operating characteristic curve, taking tied observations (mostly due to the handling of observations below the LoQ) into account and reporting the area under the curve (AUC). The maximal value of Youden's index was used to estimate the optimal cut-off point of D-lactate for the prediction of AIN. The associated sensitivity, specificity, positive predictive value, and negative predictive value for this cut-off point are reported. Positive predictive value and negative predictive value are reported with the awareness of the possible overestimated prevalence of AIN due to the inclusion of high-risk patients.

Statistical analyses were performed for all patients and the following subgroups: patients older than 70 years of age, high-risk patients, and non-high-risk patients.

Power calculation was not feasible due to the exploratory nature of the study. We included every referred patient during 1½ year, trying to maximize the number of AIN patients. The distribution of D-lactate in a general surgical population is unknown, again compromising a formal power calculation.

p Values < 0.05 were considered statistically significant.

The analysis was performed using Stata (Stata Statistical Software: Release 16; College Station, TX: StataCorp LLC.

Results

We included 2871 patients referred to the Gastrointestinal Surgical Department, Aalborg University Hospital. Additionally, we included 87 in-hospital high-risk patients suspected of suffering from AIN after this period.

The characteristics of the total study population are shown in Supplementary Table 1 (available online). We

Table 2. Diagnoses in 1479 Acute Patients.

	n	(%)	Mean D-Lactate	(SD)	% < LoQ
AIN patients	23	(1.6)	0.076	(0.084)	52
Pulmonology					
COPD	1	(0.1)	0.092	(.)	0
Hematology					
Anemia	16	(1.1)	0.060	(0.050)	44
Nephrology/urology					
Chronic renal diseases	1	(0.1)	0.025	(.)	100
Epididymitis	3	(0.2)	0.138	(0.133)	0
Dehydration	5	(0.3)	0.033	(0.017)	80
Cystitis/pyelonephritis	5	(0.3)	0.048	(0.031)	60
Kidney stone	21	(1.4)	0.036	(0.021)	76
Endocrinology					
Diabetes	40	(2.7)	0.056	(0.040)	50
Gynecology					
Endometriosis	1	(0.1)	0.058	(.)	0
Ovary diseases	1	(0.1)	0.025	(.)	100
Infectious disease					
Pneumonia/flu	6	(0.4)	0.033	(0.019)	83
Gastroenteritis	14	(0.9)	0.034	(0.018)	79
Cardiology					
Hypertension	62	(4.2)	0.052	(0.041)	56
Heart disease	114	(7.7)	0.055	(0.046)	54
Gastrointestinal medicine					
Enteritis and colitis	43	(2.9)	0.053	(0.036)	49
Functional malfunction in intestine	51	(3.4)	0.041	(0.030)	73
Non-GI cancer					
Cancer	52	(3.5)	0.045	(0.031)	63
Vascular surgery					
Peripheral vascular arterial disease	1	(0.1)	0.084	(.)	0
Gastrointestinal surgery					
Internal hernia	2	(0.1)	0.040	(0.021)	50
Observation for cancer	2	(0.1)	0.025	(0.000)	100
Intestinal volvulus	2	(0.1)	0.025	(0.000)	100
Intestinal perforation	4	(0.3)	0.059	(0.047)	50
Hepatic diseases	4	(0.3)	0.111	(0.065)	25
Gastrointestinal bleeding	6	(0.4)	0.050	(0.041)	67
Ileus	8	(0.5)	0.025	(0.000)	100
Procedure related complications	10	(0.7)	0.046	(0.032)	60
GI cancer	16	(1.1)	0.097	(0.055)	13
Other intestinal diseases	33	(2.2)	0.038	(0.022)	70
Ventricular and duodenal diseases	35	(2.4)	0.041	(0.026)	69
Pancreatitis	37	(2.5)	0.055	(0.039)	54

(*continued on next page*)

Table 2. (*continued*)

	n	(%)	Mean D-Lactate	(SD)	% < LoQ
Upper GI tract obstruction/dysphagia	42	(2.8)	0.052	(0.034)	55
Hernias	48	(3.2)	0.046	(0.043)	69
Intestinal obstruction	53	(3.6)	0.039	(0.031)	77
Cholecystitis	59	(4.0)	0.066	(0.046)	42
Diverticulitis	60	(4.1)	0.058	(0.038)	48
Upper GI ulcer w/o complications	66	(4.5)	0.039	(0.025)	71
Gallstone related disease w/o complications/procedures	72	(4.9)	0.044	(0.032)	65
Appendicitis	116	(7.8)	0.047	(0.039)	66
Other					
Perianal abscess	5	(0.3)	0.104	(0.036)	0
Abscess cutis	8	(0.5)	0.034	(0.017)	75
Observation due to suspicion of disease	30	(2.0)	0.037	(0.023)	73
Proctological disorders	39	(2.6)	0.045	(0.047)	69
Other specified circumstances	84	(5.7)	0.043	(0.032)	70
Abdominal pain	178	(12.0)	0.036	(0.032)	81

SD = standard deviation; LoQ = limit of quantification; AIN = acute intestinal necrosis; COPD = chronic obstructive pulmonary disease; GI = gastrointestinal.

identified 44 patients diagnosed intraoperatively with AIN; 37 patients were diagnosed with AIN, and seven patients were diagnosed with acute intestinal ischemia with complete intraoperative bowel recovery. The differences in arteriosclerotic disease, hypertension, and anticoagulation use were eliminated after adjusting for age and sex (Supplementary Table 2, available online). Every fourth referred patient was an active smoker, and smoking status was equally distributed among AIN patient subgroups (vascular and nonvascular AIN). AIN patients were more prone to arteriosclerotic disease and hypertension (Supplementary Table 1).

The 2914 (98.5%) non-AIN patients served as controls. The main diagnoses of the controls for the actual hospitalization are shown in Supplementary Table 3 (available online). A considerable proportion (30.2%) were patients suffering from disease entities other than those related to abdominal surgical diseases.

Abdominal explorations were performed in 538 control patients with 400 laparoscopies, of which 37 were converted to laparotomies.

D-Lactate, Cut-Off, and Lipemic Interference

D-lactate was measured in all 2958 included patients. Result of stability test for D-lactate: Suboptimal preanalytical storage conditions led to a median drop of 18% of D-lactate (90% confidence interval [CI] −25–2%), which was considered acceptable.

Preset-cut-off. The D-lactate cut-off level with respect to AIN has been reported to be in the range of 0.2–0.38 mM (13,27,28). D-lactate data were first analyzed using the cut-off of 0.3 mM, as reported by Rasmussen et al. (24). Rasmussen et al. developed the automated D-lactate method used in this study, and the tolerated L-index of 60 was reported to impose a negligible interference at this cut-off level (24). In the present study, one case and 162 controls were excluded from the analysis due to this L-index level. Only one AIN patient and four controls demonstrated a D-lactate value above 0.3 mM, corresponding to sensitivity at 0.023 and specificity at 0.999, making this cut-off level useless.

Study cut-off. This study displayed unexpectedly low levels of D-lactate; the mean D-lactate was 0.076 (SD 0.084) mM (< LoQ: 52%) in AIN patients and 0.047 (SD: 0.037) mM (< LoQ: 64%) in controls (Figure 1). The optimal cut-off point of D-lactate for the prediction of AIN in this study was estimated to be 0.0925 mM using the maximal value of Youden's index. In Rasmussen et al., lipemic interference was calculated to have an impact of 1/1200 (24). Using a limit of 20%, lipemic interference at a cut-off level of 0.0925 mM corresponding to 0.0185 mM

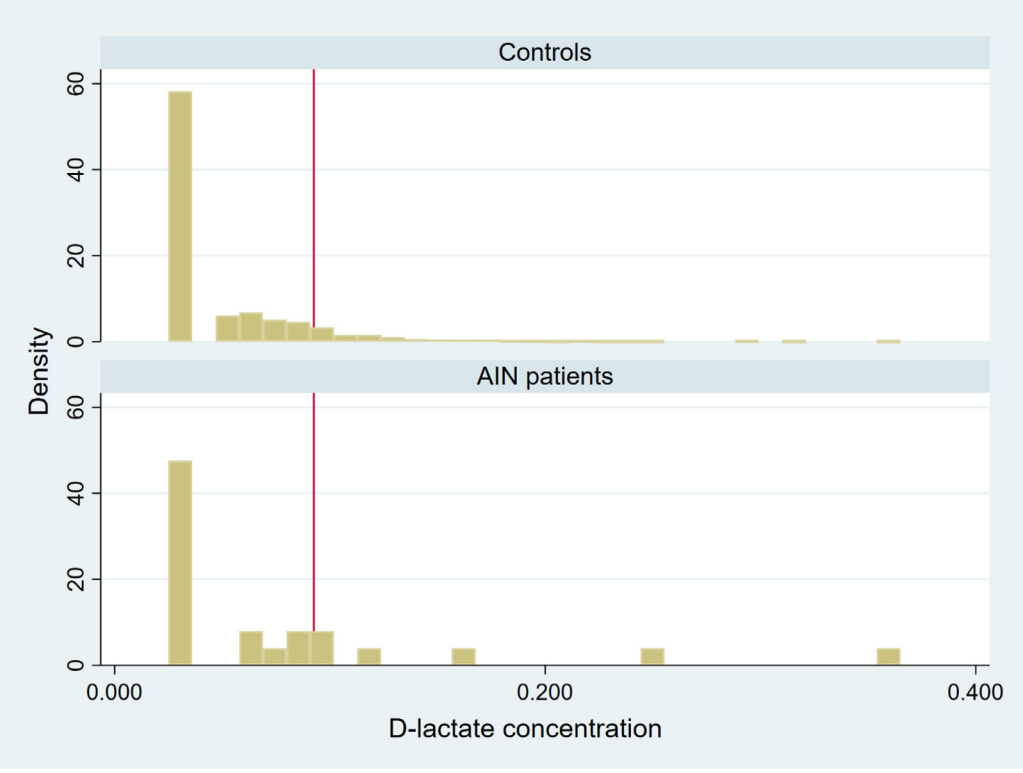

Figure 1. D-lactate concentrations (mM) in 23 acute intestinal necrosis (AIN) patients and 1456 controls. Red line: D-lactate cut-off.

results in an L-index limit of 22. Re-evaluation of the results using the L-index of 22 ensured elimination of high D-lactate measurements caused by interference. Hence, 1458 controls and 21 AIN patients were excluded from the analysis due to lipemic interference, leaving 23 cases and 1456 controls with an L-index below 22 for the analysis of the diagnostic value of D-lactate in AIN. The overall mean of D-lactate in nonexcluded patients was 0.047 mM (SD 0.039). Additionally, the mean D-lactate level in AIN patients was 0.076 mM (SD 0.084) and was not significantly different from that in controls. An overview of the patient flow is outlined in Figure 2.

The demographics (Table 1) and diagnoses (Table 2) of the remaining patients as well as the mean D-lactate measurements were comparable with those of the entire cohort prior to exclusion due to lipemic interference (data not shown). The mortality in AIN patients was significantly higher than that in controls (odds ratio 2.7; 95% CI 1.1–6.8, $p = 0.039$, adjusted for age).

Demography and surgical procedures for the 23 AIN patients are displayed in Table 3.

Diagnostic Performance of D-Lactate for AIN

The diagnostic performance for D-lactate with respect to AIN in nonexcluded patients and subgroups is displayed in Table 4. Analysis of the total study cohort showed sensitivity < 0.3 and specificity at 0.89. Restricting the analysis to patients above 70 years of age, high-risk or low-risk patients alone display similar sensitivity and specificity rates.

Discussion

A fast and reliable biomarker is crucial in the diagnostic process regarding AIN (3,29). A biomarker could potentially serve as a hallmark of AIN and help evoke the initial AIN suspicion in patients with a blurred clinical picture, and thereby, might reduce the devastating high mortality in AIN (29,30). A proposed biomarker, D-lactate, was examined in this cross-sectional study with consecutive enrollment of all adult patients who were acutely referred to a gastrointestinal surgical department and additional enrollment of high-risk patients suspected of suffering from AIN. However, significant lipemic interference caused the exclusion of approximately every second patient. Additionally, D-lactate showed insufficient diagnostic performance with a low AUC and low sensitivity in the nonexcluded patients. Thus, D-lactate measurements in this set-up left out half of the patients and failed to select AIN patients from other gastrointestinal conditions in the remaining patients. This conclusion

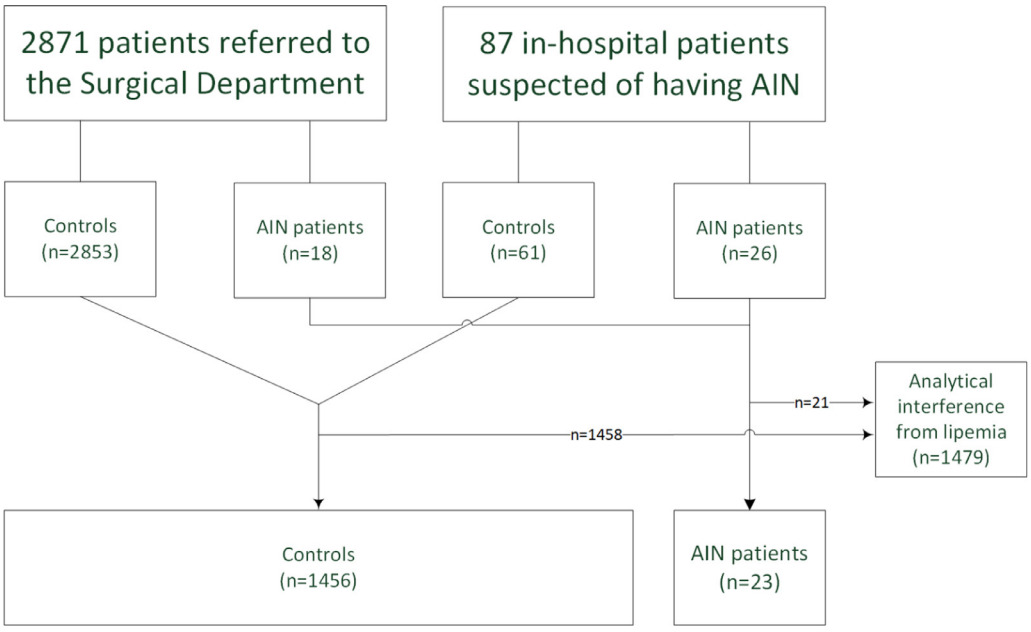

Figure 2. Flowchart for acute intestinal necrosis (AIN) patients and controls.

Table 3. Characteristics and Surgical Procedures of 23 AIN Patients.

Vascular Intestinal Necrosis	Nonvascular Intestinal Necrosis	Nonvascular Intestinal Ischemia	
Variable	n (%)	n (%)	n (%)
Patients	6 (100.0)	13 (100.0)	4 (100.0)
Male	1 (16.7)	5 (38.5)	3 (75.0)
Age above 70 years	5 (83.3)	8 (61.5)	3 (75.0)
Tree month mortality	3 (50.0)	4 (30.8)	0 (0.0)
Age, years: mean (SD)	73.7 (8.6)	77.0 (12.4)	72.9 (7.5)
Hours between blood sample and operation, hours: mean (SD)	3.5 (2.2)	11.0 (20.3)	5.1 (5.5)
Adhesiolysis	0 (0.0)	5 (38.5)	1 (25.0)
Intestinal resection	6 (100.0)	13 (100.0)	0 (0.0)
Small intestine resection	5 (83.3)	13 (100.0)	0 (0.0)
Colon resection	1 (16.7)	2 (15.4)	0 (0.0)
Stoma formation	3 (75.0)	3 (23.1)	0 (0.0)

AIN = acute intestinal necrosis; SD = standard deviation.

corresponds with some newer studies and a meta-analysis from 2013 (27,28,31,32).

The AUC of D-lactate was low but corresponds well to the studies by Shi et al. (AUC 0.69) and Hong et al. (AUC 0.506) (13,28). Our cut-off was calculated using the Youden index, but adjustment of the cut-off within the range of the literature (0.012–0.38 mM) would affect the sensitivity and specificity accordingly (13,27,28,31,33). Improving the sensitivity would be at the cost of the specificity and vice versa, which would not impact the overall conclusion.

The clinical utility of D-lactate as measured in our study was severely limited by the right-skewed distribution of D-lactate, with the majority of both AIN patients (63%) and controls (52%) displaying D-lactate levels below the LoQ (0.05 mM) and a substantial proportion below our LoD (0.04 mM) (Figure 1). Our graphical illustration showed an almost equal distribution between

Table 4. Diagnostic Performance for D-Lactate: Main Results and Subgroups.

	AIN n	Controls n	AUC (95% CI)	Sensitivity	Specificity	PPV	NPV
All patients	23	1456	0.588 (0.465–0.712)	0.261	0.892	0.037	0.987
Patients above 70 years	16	493	0.602 (0.455–0.749)	0.313	0.850	0.063	0.974
High-risk only	16	47	0.579 (0.422–0.736)	0.313	0.787	0.333	0.771
Low-risk only	7	1409	0.416 (0.226–0.606)	0.143	0.896	0.007	0.995

Cut-off value was estimated to 0.0925 mM, corresponding to a Youden index of 0.198 (SE 0.105) based on the complete cohort, and this value is used in all groups.
AIN = acute intestinal necrosis; AUC = area under the curve; PPV = positive predictive value; NPV = negative predictive value.

AIN patients and controls, and even very high levels of D-lactate over a certain limit could not differentiate AIN patients from non-AIN patients. Altogether, a diagnostic test with the AUC, sensitivity, and specificity as this D-lactate measurement is clinically useless. To our knowledge, no other study reports LoQ and LoD with respect to D-lactate analysis. Nuzzo et al. likewise report right-skewed distribution of D-lactate plasma concentration in AIN patients and controls and, in addition, reach a similar overall conclusion (31).

Lipemic interference in spectrophotometric methods was addressed by Nielsen et al. in their study in pigs (34). They reported no lipemic interference with respect to D-lactate analysis. To our knowledge, lipemic interference has not been addressed thoroughly in human studies on D-lactate, possibly due to the range of D-lactate cut-off values in the literature (0.2–0.38 mM), where lipemic interference might be of limited significance (13,24,27,28). We reported a cut-off value of 0.0925 mM and observed significant lipemic interference, hence excluding half of the study population. In a recent study, Nuzzo et al. reported an even lower cut-off at 0.012 mM (31). They used a similar spectrophotometric method without addressing lipemic interference (31). However, as in our study, they did not find differences between AIN patients and controls.

The inclusion of patients suspected to suffer from AIN—the high-risk patient group—mimics the most frequent design of prospective studies in the field (31,35,36). Restricting the analysis to high-risk patients in our study showed no statistically significant difference in the mean D-lactate concentration between AIN patients and controls when taking lipemic interference into account, and the diagnostic performance was still not adequate. Moreover, these patients were enrolled at the point of the decision for an acute operation, making D-lactate elevation irrelevant because the clinical evaluation dictated surgery, regardless of the existence of a blood-based biomarker. This emphasizes the main weaknesses of the most frequently used study design in the literature concerning D-lactate: including only *suspected* AIN patients.

A *fast* and reliable biomarker is highly needed, and the first 6 h after onset are crucial (2). D-lactate has been proposed to be an *early* biomarker for AIN (14,27). The design of this study was optimized with respect to early stages of AIN, with immediate inclusion at admission omitting a time-consuming clinical or paraclinical evaluation before enrollment. This ensured inclusion of the most difficult part of AIN patients, the unsuspected AIN patients and, theoretically, the early stages of AIN. However, the mean D-lactate in acute referred patients with an average risk of AIN (non-high-risk patients) was almost equal in the AIN group and in the controls after taking lipemic interference into account. Thus, in this study, D-lactate did not prove useful as a biomarker in the early stages of AIN. Acknowledging the small number of AIN patients, this conclusion is fragile but in accordance with a growing body of the literature (31,35,37).

A timely relation of D-lactate elevation was also explored in this study. Nevertheless, we found no relation between D-lactate level and time range prior to operation (Supplementary Figure 1, available online). In other words, we found no evidence that D-lactate at a certain level might have the potential to prompt intervention.

Due to the narrow time range of about 6 h from the AIN event to irreversible intestinal damage, the automated analytical setup was considered vital. As described, the enzymatic spectrophotometric setup was developed. Several other studies have used similar methods with positive, negative, and uncertain conclusions on D-lactate in AIN

patients (14,15,27,28,31,35,36,38,39). In contrast, one study used enzyme-linked immunosorbent assay (ELISA) in D-lactate analysis and found D-lactate useful (13). Shi et al. reports the ELISA method as rather time consuming (4 h) (13). However, with the wash procedure as a part of ELISA, the interference problem is eliminated. Nevertheless, the narrow time range favored the automated enzymatic spectrophotometric setup and was the most prevalent method in the literature. Thus, this method was adopted for this study.

Most nonvascular AIN events (e.g., hernia and bowel strangulation) can easily be diagnosed on abdominal CT scans. In contrast, vascular necrosis is the most challenging subgroup to diagnose among AIN subgroups. We had only six vascular AIN patients left for analysis after the exclusion of patients with lipemic interference, making formal statistic considerations and weighty conclusions meaningless in this subgroup. D-lactate level tended to be elevated in this small group of vascular AIN patients (0.076 mM; 95% CI 0.040–0.112) compared with controls (0.047 mM; 95% CI 0.045–0.049). Nevertheless, the benefit of a dedicated study in this subgroup is questionable due to the lipemic interference problem.

The control group included a large group of patients without abdominal diseases as well as numerous patients evaluated in the Emergency Department and discharged after the initial examination. Supplementary analysis of this group alone as a control group showed no notable difference in mean D-lactate concentration (data not shown); hence, D-lactate could not even discriminate AIN patients from patients without abdominal surgical diseases.

Recently, Nuzzo et al. illuminated the diagnostic performance of D-lactate in vascular AIN patients (31). They also rejected D-lactate as an AIN biomarker. However, AIN patients were included in their study after a CT scan and clinical evaluation. This might exclude patients suffering from early AIN displaying only vague symptoms and potentially introducing selection bias, which might explain the difference in D-lactate cut-off. In contrast, we performed a cross-sectional study including every referred acute patient, potentially also harvesting exactly this group of AIN patients with vague symptoms. Moreover, essential methodological differences (e.g., different D-lactate kit) may lead to some of the differences in D-lactate performance. In conclusion, we agree with Nuzzo et al. that the diagnostic performance of D-lactate is limited (31).

Our study supports the high mortality rates of AIN reported in the literature (1). Almost every third patient with AIN died within the first 90 days after the AIN event, compared with fewer than every 10th patient in the control group. Vascular AIN patients demonstrated the highest 90-day mortality rate among the cases by far, and after 3 months, every second vascular AIN patient was dead. This is in accordance with the mortality rate presented in the literature mentioned above.

Limitations and Strengths

Our study has some limitations that must be addressed. First, the low D-lactate cut-off and the following lipemic interference considerations resulted in the exclusion of half of the patients. It roughly halved the AIN patients and the vascular AIN patients to six and weakened the power of our conclusions.

Second, due to the low number of AIN patients in the first inclusion period, we appended an inclusion period with high-risk patients to increase the number of AIN patients. This might have resulted in an overestimation of the prevalence of AIN affecting the positive and negative predictive values. The second inclusion period introduces uncertainty about the prevalence in the cohort of non-high-risk patients, which include AIN patients with vague symptoms—the group of particular concern. However, AUC, sensitivity, and specificity were unaffected.

Third, the great number of controls compared with AIN patients might seem disproportionate. Nevertheless, D-lactate performance was insufficient, even in the stratified analysis comparing AIN patients with elderly, high-risk and low-risk patients.

Fourth, consecutive inclusion of patients was intended in this study, but due to scarcity of laboratory staff, 5 weeks of vacation, 38 public holidays, and a few more days were excluded each year. To our knowledge, thromboembolic events are not affected by short holiday seasons. However, shortening the inclusion period is a study limitation, as it potentially lowers the number of the included AIN patients.

Fifth, national legislation limits the possibility of research in children. Excluding children was assessed to be permissible due to the high median age of AIN patients in general and the extremely low frequency of AIN in children (40). Thus, excluding children from the study is considered of minor importance.

To our knowledge, this study is the largest one of D-lactate as a marker of AIN in an unselected surgical population. We consecutively included all referred patients to gather as many AIN patients as possible, especially the few unexpected patients with vague clinical symptoms of AIN. Furthermore, we displayed D-lactate ranges in a wide group of different diseases. In addition, importantly, we uncovered a substantial lipemic interference problem in spectrophotometric studies on D-lactate in AIN patients, which must be taken into consideration in all similar studies in the field. Finally, we show that even in a high-quality, large-scale, automated analytical method, the D-lactate diagnostic performance

is insufficient with respect to AIN in acute surgical patients.

Conclusion

This study demonstrated that D-lactate does not fulfill the demands of a reliable biomarker of AIN owing to its low diagnostic performance, even in a high-quality automated analytical set-up. Further research must focus on other blood-based biomarkers or series of biomarkers.

Declaration of competing interest

All authors declare that they have no conflicts of interest.

CRediT authorship contribution statement

David Straarup: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. **Kåre A. Gotschalck:** Writing – review & editing. **Peter A. Christensen:** Investigation, Methodology, Supervision, Validation, Writing – review & editing. **Rikke W. Rasmussen:** Formal analysis, Investigation, Writing – review & editing. **Henrik Krarup:** Writing – review & editing. **Søren Lundbye-Christensen:** Formal analysis, Investigation, Supervision, Writing – review & editing. **Aase Handberg:** Conceptualization, Investigation, Methodology, Supervision, Writing – review & editing. **Ole Thorlacius-Ussing:** Conceptualization, Methodology, Resources, Writing – review & editing.

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Supplementary Materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jemermed.2024.01.001.

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Article Summary

1. Why is this topic important?

Intestinal necrosis due to obstructed blood supply is challenging to diagnose in time due to unspecific clinical presentation. No reliable biomarker exists in spite of decades of research.

2. What does this study attempt to show?

D-lactate, a proposed reliable biomarker, was tested in a large-scale study in acute patients. We found low diagnostic performance for intestinal necrosis.

3. What are the key findings?

In spite of the high-quality, automated analytical method, the D-lactate diagnostic performance was insufficient with respect to intestinal necrosis. Moreover, lipemic interference excluded roughly every second patient-sample of the analysis.

4. How is patient care impacted?

The mortality for intestinal necrosis has been reported to be above 60%. Obstructed blood supply causes irreversible intestinal necrosis within 6 h. A reliable biomarker is crucial to accelerate the diagnostic process and initiate timely salvage surgery. This might ultimately save patients' lives. D-lactate is not a suitable biomarker for intestinal necrosis.