Original Research

The value of plasma neurotensin and cytokine measurement for the detection of bowel ischaemia in clinically doubtful cases: A prospective study

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Abstract

The aim of this prospective study was to examine whether serum neurotensin, interleukin (IL)-6, and IL-8 are early predictor of bowel ischaemia especially in clinically equivocal cases. To this end, 56 patients were assigned to the following groups according to their disease: bowel ischaemia (group 1: $n = 14$), small bowel obstruction (group 2: $n = 12$), acute inflammation (group 3: $n = 6$), perforation (group 4: $n = 8$), and colorectal adenocarcinoma (group 5: $n = 16$). Fifteen healthy controls were assigned to group 6. Blood samples were obtained at enrollment, all measurements were done blindly, and all patients underwent surgery. Pretreatment doubtful diagnosis comprised of ileus, mild abdominal pain, and indeterminate imaging. Blood urea nitrogen, lactic acidosis, diagnostic workup, and IL-6 were predictors of diagnosis in univariate analysis. In multivariate analysis, IL-6 ($P < 0.001$) and diagnostic workup ($P < 0.01$) were independent predictors of the definite diagnosis. Neurotensin and IL-8 did not differentiate among groups. Considering clinically doubtful cases, IL-6 perfectly differentiates mesenteric ischaemia (of infarction/embolic/occlusive aetiology) from the rest of the indeterminate pathologies. The optimum cut-off point for IL-6 was 27.66 pg/mL. The value of serum IL-6 (27.66 pg/mL) had sensitivity = 1 and specificity = 1. In conclusion, plasma IL-6 measurement on admission might be an additional diagnostic tool that can predict bowel ischaemia in doubtful clinical situations.

Keywords: acute abdomen, bowel obstruction, IL-6, IL-8, mesenteric ischaemia, neurotensin

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Introduction

Mesenteric ischaemia continues to be an extremely morbid disease. Mortality varies from 30% to 90%, depending on the pathogenesis,[1–3] and may actually be even higher if undiagnosed mesenteric ischaemia is taken into account. The diagnosis of acute mesenteric ischaemia is often one of exclusion and is only established in one-third of patients before operation or death.[3]

Laboratory studies are not specific for mesenteric ischaemia and the information from imaging modalities has a low sensitivity and a number of limitations.[1,4] Additionally, angiography is an invasive procedure not readily available on an emergency basis.

The intestinal mucosa is highly sensitive to ischaemia,[5] suffering early impairment of cellular permeability and transcapillary barrier function. The result is a loss of the barrier function of the gut, translocation of bacteria, and systemic endotoxin release. In fact, ischaemia secondary to haemorrhage alone, without detectable endotoxin in the portal circulation, increases cytokine gene expression. Furthermore, gut hypoxaemia itself, in the absence of blood loss or tissue injury, induces pro-inflammatory cytokines which contribute to this syndrome.

There is also an increase in cytokine production by Kupffer cells following hypoxia to intestinal cells. Kupffer cells are the primary source of amplification of pro-inflammatory cytokine release following intestinal ischaemia. Kupffer cells are responsible for the increased levels of tumour necrosis factor alpha (TNF-α) and interleukin (IL)-6 in trauma, haemorrhagic shock, and resuscitation.[6] While gut ischaemia is a potent stimulant of systemic inflammatory response syndrome (SIRS) and Kupffer cells are a major

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source of cytokines central to the cascade events,[6] the gut–liver axis and associated cytokines have been the primary source of knowledge about intestinal ischaemia.

The highest concentration of neurotensin is in the jejuno-ileal segment of small bowel, but it has also been identified in the oesophagus, stomach, duodenum, and large bowel.[7] Substantial evidence indicates that stored neurotensin can be rapidly released during a variety of physiological and pathological conditions including the modulation of intestinal responses to stressful and inflammatory insults.[8] Notably, neurotensin and the neurotensin S1 receptor have been linked to stress responses.[9]

The present study was aimed at answering the following question: is the patient at some stage of the process of bowel obstruction with the appropriate response being to wait-and-see, or does the patient have early stage mesenteric ischaemia with/out bowel obstruction and need an emergency operation?

Materials and methods

Patients

During a four-month period, from October 2011 to February 2012, 56 blood samples were collected from patients presenting with an acute abdomen for serum neurotensin, IL-6, and IL-8 measurement. Preoperative workup included clinical evaluation, haematological and biochemical parameters, and abdominal imaging.

Patients were considered for analysis after the final histologic report and/or theatre records. The patients were divided into six categories based on the final diagnosis: bowel ischaemia (group 1), small bowel obstruction (group 2), inflammatory diseases (group 3), perforated viscous (group 4), and colorectal adenocarcinoma (group 5). Additionally, 15 blood samples from healthy control subjects with a recent colonoscopy (group 6) were collected.

The time from admission to theatre was left to the attending surgeon.

The methodology followed was firstly to disclose the independent factors predicting cause and pathophysiology of an acute abdomen, and secondly to investigate their role in predicting mesenteric ischaemia before this becomes clinically or radiologically apparent. For this latter step cases with equivocal preoperative diagnosis were analysed separately.

The study was approved by the Institutional Review Board and informed consent was obtained from each patient.

Patients were included when presenting with acute abdomen and age was over 18, when the cause and pathophysiology of the abdominal diseases considered both the small and large bowel, and when there was no need for emergency operation.

Patients were excluded when they did not undergo an operation, when a definite histologic diagnosis was not possible, when they presented with lactic acidosis/sepsis/multiorgan failure, when peritonitis was due to other causes than perforation, when mesenteric ischaemia was documented by enhanced computed tomography (CT), and when they had not formally indicated their informed consent.

A clinically equivocal diagnosis was assigned using the following criteria: (a) history: non-specific abdominal pain up to 12 h before admission; (b) physical examination: bowel dilatation with interruption of normal peristaltic activity, clinically suspicious for mesenteric ischaemia; (c) white blood cell (WBC): within reference range or mild leucocytosis and/or leftward shift; (d) ABG: absence of metabolic acidosis/lactate; (e) plain abdominal films: ileus, small bowel obstruction, edematous/thickened bowel walls, and paucity of gas in the intestines; (f) CT scan: excluding other causes of abdominal pain, mild bowel wall and/or mesenteric oedema, abnormal gas patterns; (g) CT angiography: no pathognomonic findings.

Plasma neurotensin and cytokine measurement

All samples were obtained on the day of admission and after centrifugation at 3000 rpm for 10 min they were stored at −80°C until assayed. Serum and supernatant levels of neurotensin, IL-6, and IL-8 were measured using an enzyme-linked immunosorbent assay (ELISA). The neurotensin and cytokine measurements were blinded and were performed as follows:

Neurotensin. A competitive enzyme immunoassay (EIA) kit was used for detecting neuropeptide Y peptide according to the manufacturer's instructions (RayBio Neuropeptide Y EIA). The detection range was 0.1–1000 ng/mL with sensitivity expressed as minimum detectable concentration of neuropeptide Y at 180 pg/mL or 16.59 pmol/L.

IL-6. Serum IL-6 levels were measured by the IL-6 ELISA method (human IL-6 ELISA kit Orgenium Laboratories, Vantaa, Finland) according to the manufacturer's instructions. The limit of detection of the assay was 7.8–500 pg/mL with specificity that recognizes both natural and recombinant human IL-6 and sensitivity less than 2 pg/mL.

IL-8. The serum IL-6 levels was measured by the IL-8 ELISA method according to the manufacturer's instructions. The limit of detection was 3.9–125 pg/mL with specificity that recognizes both natural and recombinant human IL-8 and sensitivity less than 2 pg/mL.

Statistical analyses

The Mann-Whitney *U* test was used in order to test the significance of the difference between two independent samples of an ordinal variable, as well as differences in the shapes of the distributions (not just the locations of the ranks) of the two groups.

In univariate analysis of two tests were used: (a) Kruskal-Wallis analysis of variance (ANOVA) and median test when the groups (coding variable) were to be compared by selected dependent variables and (b) standard Pearson product moment correlation coefficient (*r*) to measure the extent to which two variables were related.

The least square methods of the general linear model was used to estimate and test hypotheses about effects. The general linear model was applied to analyse an ANOVA design with both categorical and continuous predictor variables in multivariate analysis.

The Bonferroni procedure was used to adjust the significance levels for the individual *post hoc* comparisons.

A receiver–operating characteristic curve was used to summarize the performance of a two-class classifier across the range of possible thresholds.

Sample size for an unpaired two sample Student's *t*-test was used in order to validate our results in cases suspicious for mesenteric ischaemia.

Significance was considered at a level <0.05. Statistical version 7 (StatSoft, Inc., www.statsoft.com, Tulsa, OK, USA) was used for data analysis.

Results

Results according to different pathology

Fifty-six patients were assigned to the following groups according to their disease based on theatre records and histology report: bowel ischaemia (group 1: $n=14$), small bowel obstruction (group 2: $n=12$), inflammatory diseases (group 3: $n=6$), perforated viscus (group 4: $n=8$), colorectal adenocarcinoma (group 5: $n=16$). Fifteen healthy controls were assigned to group 6 (Table 1). During the study period, 18 patients were excluded (high lactic acid levels/sepsis: $n=6$, peritonitis due to other causes than perforation: $n=8$, mesenteric ischaemia, documented by enhanced CT: $n=2$, and informed consent not signed: $n=2$). There was no statistically significant differences in clinical and biochemical parameters between patients and controls (Table 2).

Among the analysed parameters, only blood urea nitrogen (BUN), lactic acidosis, diagnostic workup, and IL-6 were predictors of the definite diagnosis in univariate analysis (Table 3). In multivariate analysis (including only the parameters that gained significance in univariate analysis), IL-6 ($P<0.001$) and diagnostic workup ($P<0.01$) were independent predictors of the definite diagnosis.

Neurotensin and IL-8 did not differentiate among the groups. The descriptive statistics of IL-6, neurotensin, and IL-8 measurements among the different disease entities and healthy individuals are presented in Table 4.

Considering our entire patient/control cohort, *post hoc* comparisons using the Bonferroni test revealed that IL-6 perfectly differentiates mesenteric ischaemia from bowel

Table 2 Clinical and biochemical parameters of patients and controls

Clinical/biochemical parameters	Patients	Controls	*P* value
Age (years)	67.9 ± 15.7	63.75 ± 19.46	0.352
M/F	36/20	10/5	0.894
BMI (kg/m^2)	26.69 ± 5.31	26.65 ± 4.12	0.974
Creatinine (mg/dL)	1.07 ± 0.47	0.96 ± 0.31	0.351
ALT (U/L)	25.49 ± 25.65	18.3 ± 4.39	0.059
AST (U/L)	16.44 ± 47.79	16.95 ± 7.48	0.422
GGT (U/L)	31.52 ± 45.02	26.55 ± 26.39	0.646
ALP (U/L)	66.35 ± 41.51	67.7 ± 25.67	0.892

Table 1 Patients were assigned to five groups according to the cause and pathophysiology of their disease which was documented by the final histologic report and/or theatre records[a]

Group	Cause and pathophysiology of the abdominal diseases	N	Definite diagnosis	Equivocal clinical diagnosis
Group 1	Vascular/bowel ischaemia	14	10: mesenteric ischaemia due to infarction/thrombosis 4: small bowel strangulation/necrosis	8: mesenteric ischaemia (aetiology 5: thrombo-embolic, 3: occlusive)
Group 2	Mechanical	12	12: small bowel obstruction	4: small bowel obstruction
Group 3	Inflammatory diseases	6	3: Crohn's disease 1: appendicitis 1: mesenteric lymphadenopathy 1: sclerosing mesenteritis	1: undiagnosed Crohn's disease with small bowel obstruction
Group 4	Perforated viscus	8	3: sigmoid diverticulum 1: rectal perforation 1: duodenal ulcer 3: small bowel perforation	_
Group 5	Colorectal adenocarcinoma	16	8: rectal 2: sigmoid 4: ceacum 1: transverse colon 1: left colic flexure	_
Group 6	Control	15	Healthy individuals paired by gender, age, BMI, and biochemical parameters	_
Total		71		13

[a]Last column presents cases with equivocal preoperative diagnosis and the definite postoperative diagnosis.

Table 3 Univariate and multivariate analysis for the prediction of the definite patient diagnosis

Parameter	Univariate analysis *P* value	Multivariate analysis *P* value
Age	0.157	–
BMI	0.276	–
WBC	0.079	–
BUN	<**0.05**	NS
Creatinine	0.319	–
ALT	0.207	–
AST	0.247	–
GGT	0.549	–
ALP	0.088	–
Bil-D	0.195	–
Neurotensin	0.363	–
IL-6	<**0.0001**	<**0.0001**
IL-8	0.321	–
Gender	0.232	–
Lactic acidosis	<**0.05**	NS
Smoking	0.707	–
Alcohol	0.630	–
Cardiovascular history	0.871	–
Respiratory system history	0.891	–
Kidney/urinary history	0.139	–
Workup	<**0.01**	<**0.01**

Bold values are significantly different.

Table 4 Descriptive statistics of neurotensin, IL-6, and IL-8 measurements among the different disease entities and healthy individuals

	Mean	Standard error	+95%	−95%
Neurotensin (pg/mL)				
Ischaemia	32,077	15,852	0.321	63,833
Perforation	29,198	16,814	−4,485	62,880
Adenoca large bowel	48,110	12,710	22,648	73,571
Small bowel obstruction	43,294	17,975	7,286	79,302
Acute inflammation	47,015	21,268	4,410	89,621
Control	97,263	11,534	74,157	120,369
Interleukin-6 (pg/mL)				
Ischaemia	285,638	50,453	184,568	386,708
Perforation	325,746	53,514	218,545	432,947
Adenoca large bowel	42,699	40,453	−38,337	123,735
Small bowel obstruction	8,719	57,209	−105,884	123,322
Acute inflammation	336,972	67,690	201,372	472,572
Control	2,852	36,710	−74,320	72,759
Interleukin-8 (pg/mL)				
Ischaemia	103,342	14,323	74,649	132,035
Perforation	29,564	15,192	−0.870	59,998
Adenoca large bowel	30,344	11,484	7,338	53,350
Small bowel obstruction	34,230	16,241	1,695	66,765
Acute inflammation	49,713	19,217	11,217	88,209
Control	9,554	10,422	−11,324	30,431

obstruction ($P = 0.017$), large bowel cancer ($P = 0.012$), and healthy controls ($P < 0.001$; Table 5).

By contrast, IL-6 did not differentiate mesenteric ischaemia from perforation and inflammatory disease.

Clinically doubtful cases

Of the 13 patients with equivocal diagnosis (ileus plus mild abdominal pain and indeterminate imaging) in which the attending surgeon could not exclude bowel ischaemia on admission, eight patients had mesenteric ischaemia due to infarction/thrombosis/occlusion, four patients had mechanical obstruction, and one patient had undiagnosed Crohn's disease with mechanical obstruction (Table 1).

Statistical significances were observed between plasma values of IL-6 ($P = 0.001$) and IL-8 ($P = 0.006$) in the comparison between mesenteric ischaemia and the remaining cases. Neurotensin failed to gain significance ($P = 0.107$, Bonferroni *post hoc* test $P = 1.0$).

Considering clinically doubtful cases, *post hoc* comparisons revealed that IL-6 perfectly differentiates mesenteric ischaemia from the rest of the indeterminate pathology (mean 292.87 pg/mL versus mean 7.057 pg/mL, respectively, $P = 0.018$; Table 6). IL-8 also differentiates mesenteric ischaemia from the remaining pathology (mean 97.37 pg/mL versus mean 4.8 pg/mL, respectively, $P = 0.027$).

The receiver operating characteristic (ROC) curves of IL-6 and IL-8 for the detection of mesenteric ischaemia in doubtful cases are depicted in Figure 1. The optimum cut-off point selected for IL-6 was 27.66 pg/mL. The value of plasma IL-6 (27.66 pg/mL) at enrollment had sensitivity (95% confidence interval [CI]) = 1 (0.63–1), specificity (95% CI) = 1 (0.48–1), and Wilcoxon estimate of area under ROC curve = 1 in predicting mesenteric ischaemia.

The optimum cut-off point selected for IL-8 was 14.35 pg/mL. The value of plasma IL-8 (14.35 pg/mL) at enrollment had sensitivity (95% CI) = 0.875 (0.47–1), specificity (95% CI) = 1 (0.48–1), and Wilcoxon estimate of area under ROC curve = 0.95 in predicting mesenteric ischaemia.

In order to validate our results, we performed an unpaired two sample Student's *t*-test: with alpha = 0.05, power = 0.8, difference between means = 285 pg/mL, standard deviation = 236 pg/ml, degrees of freedom = 24, and a control per experimental subject ratio = 0.625 the estimated minimum sample size of cases with clinically doubtful diagnosis would be 16 cases of mesenteric ischaemia and 10 cases of bowel obstruction.

Discussion

In cases of uncertain diagnosis presenting with ileus, mild abdominal pain, and indeterminate imaging, the fundamental issue for an individual surgeon to address is whether the patient has to be operated urgently or undergo a surveillance period with conservative management. Mesenteric ischaemia has a quick progression, because collateral blood supply is limited with acute occlusion. The acuteness of the natural disease history in combination with common delays in diagnosis leads to a high mortality

Table 5 *Post hoc* comparisons revealed that IL-6 differentiates mesenteric ischaemia from small bowel obstruction, bowel cancer and healthy controls (Bonferroni test; variable IL-6: probabilities for *post hoc* tests error: between MS = 22,910, df = 56.000; mean IL-6 values for each group are depicted on first row)

Group	Ischaemia (285.64) pg/mL	Perforation (325.75) pg/mL	Adenoca large bowel (42.699) pg/mL	Small bowel obstruction (8.718) pg/mL	Acute inflammation (336.97) pg/mL	Control (2.852) pg/mL
Ischaemia		1.000	**0.012**	**0.017**	1.000	**0.001**
Perforation	1.000		**0.003**	**0.005**	1.000	**0.000**
Adenoca large bowel	**0.012**	**0.003**		1.000	**0.012**	0.997
Small bowel obstruction	**0.017**	**0.005**	1.000		**0.013**	1.000
Acute inflammation	1.000	1.000	**0.012**	**0.013**		**0.002**
Control	**0.001**	**0.000**	0.997	1.000	**0.002**	

Bold values are significantly different.

Table 6 *Post hoc* comparisons revealed that IL-6 differentiates perfectly ($P = 0.018$) and IL-8 at a lesser degree ($P = 0.027$) mesenteric ischaemia from the rest of the indeterminate pathology

Bonferroni test; variable IL-6 Probabilities for *post hoc* tests error: between MS = 35,626, df = 11.000		
Definite diagnosis	**IL-6 mean ischaemia (292.87 pg/mL)**	**IL-6 mean bowel obstruction/no ischaemia (7.057 pg/mL)**
Ischaemia	–	0.018
Doubtful cases	0.018	–
Bonferroni test; variable IL-8 Probabilities for *Challenges of Caregivinpost hoc* tests error: between MS = 4083.2 df = 11.000		
Definite diagnosis	**IL-8 mean ischaemia (97.37 pg/mL)**	**IL-8 mean bowel obstruction/no ischaemia (4.8 pg/mL)**
Ischaemia		0.027
Doubtful cases	0.027	

rate, which is around 70% for visceral emboli in a review of published reports.[3] Our results imply that IL-6 is a powerful independent predictor of the pathophysiology of abdominal disease, and moreover is a decisive tool to differentiate mesenteric ischaemia in clinically doubtful cases.

To our knowledge this is the first study focusing on the role of IL-6 and IL-8 in mesenteric ischaemia due to infarction, embolic, and occlusive aetiologies. Only two studies addressing the role of IL-6 in bowel strangulation (not in mesenteric ischaemia of infarction or embolic pathophysiology) have been reported.[10,11] Both studies found that plasma IL-6 and lactic acid levels at enrollment were predictive factors for strangulation; however, lactic acid was not an independent predictor in our study. This difference can be possibly attributed to the fact that our patients were operated on early in the course of the disease process.

Elevated lactate levels are an expression of ongoing anaerobic metabolism, suggestive of an ischaemic process. Like most other laboratory tests that may be used for the evaluation of a patient who has abdominal pain, it is not specific for mesenteric ischaemia.[12] We believe that by the time lactic acid is elevated a clinical judgment for operational management has already been made, rendering its use pointless.

The rational of assigning patients in five groups (five major categories of cause and pathophysiology of abdominal disease) at first instance was to establish the role of neurotensin, IL-6, and IL-8 as independent predictors of these categories and to delineate the respective differences between patients and healthy controls. Secondly, and after refinement of the diagnostic workup, we proceeded to validate the plasma values of the measured substances on admission as independent predictors to differentiate between the need for an emergency intervention or a wait-and-see policy.

Zhao *et al*.[9] disclosed the cellular mechanism of neurotensin-mediated pro-inflammatory intestinal effects and demonstrated that neurotensin and neurotensin S1 receptor interactions in non-transformed human NCM460 colonic epithelial cells transfected with NTS1 promoted formation of the pro-inflammatory cytokine IL-8. Contrary to the aforementioned research background, the plasma neurotensin concentrations at admission did not differentiate cases of bowel or mesenteric ischaemia (P value in univariate analysis = 0.363). Considering cases of doubtful clinical diagnosis, only neurotensin has also failed to discriminate between cases of mesenteric ischaemia and bowel obstruction without ischaemia (Mann-Whitney U test $P = 0.107$, Bonferroni *post hoc* test $P = 1.0$).

Several studies have proposed that, in the course of bowel inflammation, nerves and hormones modify central intestinal operations such as motility and transport,

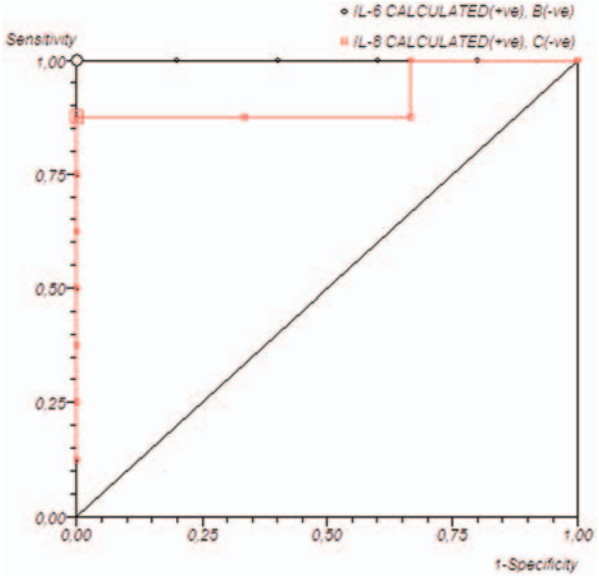

Figure 1 The ROC curves of IL-6 and IL-8 for the detection of mesenteric ischaemia in doubtful cases are shown. The value of serum IL-6 (27.66 pg/mL) at enrollment had sensitivity (95% CI) = 1 (0.63–1), specificity (95% CI) = 1 (0.48–1), and Wilcoxon estimate of area under ROC curve = 1 in predicting mesenteric ischaemia. The ROC curve line is depicted by black circles. The value of serum IL-8 (14.35 pg/mL) at enrollment had sensitivity (95% CI) = 0.875 (0.47–1), specificity (95% CI) = 1 (0.48–1), and Wilcoxon estimate of area under ROC curve = 0.95 in predicting mesenteric ischaemia. ROC curve line is depicted by red squares. (A color version of this figure is available in the online journal)

permeability, fluid secretion, and release of pro-inflammatory cytokines.[13,14] IL-8 was not found to be an independent predictor of the definite patient diagnosis (univariate analysis, $P = 0.321$), albeit in doubtful cases could discriminate well (serum value = 14.35 pg/mL) between cases of mesenteric ischaemia and bowel obstruction without ischaemia ($P = 0.027$ with sensitivity = 0.875 and specificity = 1).

Clinical and experimental data now available suggest that a given inflammatory stimulus does not result in the induction of a single mediator, but rather results in a complex cascade of cytokine release. TNF-α also triggers the production of other cytokines, which amplify and propagate its biological effects. We have not opted to measure TNF-α since this was not detected in the plasma in the majority of patients, and TNF-α levels did not correlate with bowel strangulation.[11]

IL-6 has recently been implicated as an additional mediator of the acute host response to haemorrhage, trauma, and sepsis. Shatila *et al.*[15] found that the classic signs of a gangrenous process were absent in a great proportion of patients. Among 50 patients with strangulation obstruction, fever was absent in 70% of cases, leucocytosis in 42%, and tachycardia in 42%. Many studies have been performed investigating the impact of biochemistry in mesenteric ischaemia, but no single enzyme or combination of enzymes has proved to be sensitive and specific enough to enable the diagnosis of bowel ischaemia to be made sufficiently early to alter morbidity and mortality rates.[16,17]

There is conflicting evidence in the literature regarding the value of age and WBC counts as independent predictors of mesenteric ischaemia. Bizer *et al.*[18] concluded that early surgery should be considered in patients older than 70 years of age and patients with a high WBC count. On the contrary, we have found that these parameters did not correlate.

IL-6 is a pro-inflammatory cytokine produced by monocycle phagocytes in response to a variety of stimuli, including TNF-(a. IL-6 contributes to the development of systemic inflammatory response syndrome (SIRS) via the activation of neutrophils and up-regulation of endothelial intercellular adhesion molecule 1 (ICAM-1).19,20

IL-6 did not differentiate between mesenteric ischaemia from perforation and inflammatory disease (Table 5). All eight cases of perforation and five of six cases of inflammatory disease had been diagnosed by preoperative CT scan. Preoperative workup only failed to diagnose one patient of group 3, which was a case of Crohn's disease with small bowel obstruction. In this respect, we consider the IL-6 value on admission and CT scans as complementary examinations. IL-6 and workup were independent predictors in multivariate analysis.

IL-6 perfectly differentiates mesenteric ischaemia from the rest of the indeterminate pathology (mean 292.87 pg/mL versus mean 7.057 pg/mL, respectively, $P = 0.018$). The value of serum IL-6 (27.66 pg/mL) at enrollment had sensitivity = 1, specificity = 1, and Wilcoxon estimate of area under ROC curve = 1 in predicting mesenteric ischaemia.

Potential limitations of our study are: (a) it is underpowered since half of the estimated minimum sample size of clinically doubtful diagnosis cases were enrolled, (b) due to the small number of patients, infarction/thrombosis and occlusive mesenteric ischaemia cases were not analysed separately, and (c) it has to be validated prospectively, that is to proceed or not to operative management according to plasma IL-6 concentrations on patient's admission.

In conclusion, plasma IL-6 is a sensitive predictor of mesenteric ischaemia independently of its aetiology. Moreover, it seems to be a decisive tool for clinical judgment between interventional and observational management in presentations where the diagnosis is uncertain but suspicious for mesenteric ischaemia.

Author contributions: GS analysed the results and wrote the paper; AP performed the experiments, analysed the results and wrote the paper; ChrK performed the experiments; MVK, SL, ChlK and CoK analysed the results; and AGP designed the experiments, analysed the results and wrote the paper.

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REFERENCES

1. Kumar S, Sarr MG, Kamath PS. Mesenteric venous thrombosis. *N Engl J Med* 2001;**345**:1683–8

2. Mamode N, Pickford I, Leiberman P. Failure to improve outcome in acute mesenteric ischaemia: seven-year review. *Eur J Surg* 1999;**165**:203–8
3. Schoots IG, Koffeman GI, Legemate DA, Levi M, van Gulik TM. Systematic review of survival after acute mesenteric ischaemia according to disease aetiology. *Br J Surg* 2004;**91**:17–27
4. Kazmers A. Operative management of chronic mesenteric ischemia. *Ann Vasc Surg* 1998;**12**:299–308
5. Grootjans J, Lenaerts K, Derikx JP, Matthijsen RA, de Bruine AP, van Bijnen AA, van Dam RM, Dejong CH, Buurman WA. Human intestinal ischemia-reperfusion-induced inflammation characterized: experiences from a new translational model. *Am J Pathol* 2010;**176**:2283–91
6. Tamion F, Richard V, Lyoumi S, Daveau M, Bonmarchand G, Leroy J, Thuillez C, Lebreton JP. Gut ischemia and mesenteric synthesis of inflammatory cytokines after hemorrhagic or endotoxic shock. *Am J Physiol* 1997;**273**:G314–21
7. Carraway R, Leeman SE. Characterization of radioimmunoassayable neurotensin in the rat. Its differential distribution in the central nervous system, small intestine, and stomach. *J Biol Chem* 1976;**251**:7045–52
8. Pothoulakis C, Castagliuolo I, Leeman SE. Neuroimmune mechanisms of intestinal responses to stress. In: Crafford LJ, McCann SM, Sternberg EM, Chrousos GP, Gold PW, Smith CC (eds). *Neuroimmunomodulation Molecular Integrative Systems and Clinical Advances*. NY, USA: New York Academy of Sciences, 1998:635–48
9. Zhao D, Keates AC, Kuhnt-Moore S, Moyer MP, Kelly CP, Pothoulakis C. Signal transduction pathways mediating neurotensin-stimulated interleukin-8 expression in human colonocytes. *J Biol Chem* 2001;**276**:44464–71
10. Firoozmand E, Fairman N, Sklar J, Waxman K. Intravenous interleukin-6 levels predict need for laparotomy in patients with bowel obstruction. *Am Surg* 2001;**67**:1145–9
11. Yamamoto T, Umegae S, Kitagawa T, Matsumoto K. The value of plasma cytokine measurement for the detection of strangulation in patients with bowel obstruction: a prospective, pilot study. *Dis Colon Rectum* 2005;**48**:1451–9
12. Oldenburg WA, Lau LL, Rodenberg TJ, Edmonds HJ, Burger CD. Acute mesenteric ischemia: a clinical review. *Arch Intern Med* 2004;**164**:1054–62
13. Collins SM. Stress and the Gastrointestinal Tract IV. Modulation of intestinal inflammation by stress: basic mechanisms and clinical relevance. *Am J Physiol Gastrointest Liver Physiol* 2001;**280**:G315–8
14. Pothoulakis C, Lamont JT. Microbes and microbial toxins: paradigms for microbial-mucosal interactions II. The integrated response of the intestine to Clostridium difficile toxins. *Am J Physiol Gastrointest Liver Physiol* 2001;**280**:G178–83
15. Shatila AH, Chamberlain BE, Webb WR. Current status of diagnosis and management of strangulation obstruction of the small bowel. *Am J Surg* 1976;**132**:299–303
16. Graeber GM, Cafferty PJ, Reardon MJ, Curley CP, Ackerman NB, Harmon JW. Changes in serum total creatine phosphokinase (CPK) and its isoenzymes caused by experimental ligation of the superior mesenteric artery. *Ann Surg* 1981;**193**:499–505
17. Thompson JS, Bragg LE, West WW. Serum enzyme levels during intestinal ischemia. *Ann Surg* 1990;**211**:369–73
18. Bizer LS, Liebling RW, Delany HM, Gliedman ML. Small bowel obstruction: the role of nonoperative treatment in simple intestinal obstruction and predictive criteria for strangulation obstruction. *Surgery* 1981;**89**:407–13
19. Frank PG, Lisanti MP. ICAM-1: role in inflammation and in the regulation of vascular permeability. *Am J Physiol Heart Circ Physiol* 2008;**295**:H926–7
20. Towfigh S, Heisler T, Rigberg DA, Hines OJ, Chu J, McFadden DW, Chandler C. Intestinal ischemia and the gut-liver axis: an in vitro model. *J Surg Res* 2000;**88**:160–4

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