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Urine intestinal fatty acid-binding protein predicts acute mesenteric ischemia in patients

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ABSTRACT

Background: Acute mesenteric ischemia (AMI) has a high morbidity and mortality and often presents as a diagnostic challenge. Currently, there is no blood, urine, or radiologic tests that provide a definitive diagnosis of AMI. The aim of this study was to evaluate the clinical accuracy of urine intestinal fatty acid-binding protein (I-FABP) to diagnosis AMI.

Materials and methods: Twenty patients referred to the Acute Care Surgery service at University of Alberta Hospital with suspected AMI taken to the operating room for definitive diagnosis were recruited. Pathologic findings from surgical specimens confirmed a gold standard diagnosis for intestinal ischemia. The patients found to be nonischemic became the internal controls. Conventional clinical markers were examined in blood including white blood cell count, lactate, and creatinine. Blood was also examined by enzyme-linked immunosorbent assays (ELISAs) for I-FABP and interleukin-6. Urine was examined preoperatively and 6 and 24 h postoperatively for I-FABP.

Results: Thirteen patients were pathologically diagnosed with AMI while five patients were nonischemic; two were excluded due to missing biologic specimens. There was no difference in age or gender between ischemic and nonischemic patients (56 ± 5 *versus* 66 ± 11 years old, respectively; six females with ischemic and three females in the nonischemic group). There was no difference in serum lactate and creatinine between the two groups. Serum interleukin-6 levels in patients with AMI were significantly higher than nonischemic controls (0.4 ± 0.2 ng/mL *versus* 0.2 ± 0.07 ng/mL, respectively, $P = 0.03$). There was a nonstatistically significant increase in serum I-FABP in AMI patients compared to internal controls (9 ± 3 ng/mL *versus* 2.4 ± 0.9 ng/mL, respectively, $P = 0.2$). Urine I-FABP was significantly higher in patients diagnosed with AMI than in controls (7 ± 1 ng/mL *versus* 2 ± 1 ng/mL, respectively, $P = 0.007$). The receiver operating characteristic curve illustrated that urine I-FABP discriminates significantly between patients with AMI and controls (area under receiver operating characteristic = 0.88, $P = 0.03$).

Conclusions: The traditional clinical markers lactate and white blood cell count were not able to differentiate AMI from nonischemic bowel. However, we found that urine I-FABP was a noninvasive biomarker with high specificity and sensitivity for accurately diagnosing AMI in patients. A noninvasive accurate tool for AMI would facilitate for a rapid treatment, while preventing unnecessary surgical interventions in high-risk patient populations.

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Introduction

Acute mesenteric ischemia (AMI) broadly affects patients in the areas of trauma, transplantation, cardiac surgery, and the critical ill.[1,2] It can result in a potentially fatal intestinal ischemia due to blockage of arterial or venous blood flow. Due to its elusive presentation, a high degree of clinical suspicion is important for early and accurate diagnosis of this condition. The mortality rate for AMI ranges from 60% to 80%, with some reports indicating increased incidence rates in the elderly population.[3,4] The high mortality rate for intestinal ischemia is due to the severity of the disease and, more importantly, the failure of timely diagnosis. Currently, there is no blood, urine, or radiologic tests that can provide a definitive diagnosis of AMI. A delay or missed diagnosis can lead to an irreversible intestinal necrosis resulting in a massive resection. Even a delay of 24 h in diagnosis has been shown to decrease survival rates by 20%.[5] Rapid and accurate diagnosis of AMI is essential for allowing more directed treatment modalities, either surgical or nonsurgical, and ultimately improving the prognosis. Conventional biochemical markers of intestinal ischemia such as serum lactate and white blood cell count (WBC) lack sensitivity and specificity. Invasive diagnostic tests, such as computer tomography (CT) or angiography also lack accuracy and expose already compromised patients to risk of contrast-induced nephropathy.[6] In fact, a recent article showed that 15% of patients who were thought to have ischemia on a CT scan showed normal endoscopic findings.[7]

The intestinal mucosa is highly sensitive to ischemia. Early reduction or low blood flow to the intestine can result in impairment of cellular and intestinal permeability. Due to the arteriolar architecture of the villi, prolonged ischemic insult or reperfusion injury can result in cellular dysfunction and cellular death initiating at the mucosal villous tip to the crypt.[8] This loss of cellular integrity lends itself to loss in the intestinal barrier resulting in the translocation of gut bacteria. In addition, production of oxygen-derived free radicals, proinflammatory cytokines, and other intestinal-derived factors create a self-perpetuating cascade that has the potential to escalate into a vicious cycle of continuously increasing intestinal permeability and subsequent systemic release of endotoxins and septic shock. In fact, far too often diagnosis of intestinal ischemia does not occur until the patients display clinical symptoms of sepsis.[2,9,10]

Organ-specific biomarkers are highly coveted because they provide easily measurable and specific diagnostic criteria for identifying patients needing further care. Intestinal fatty acid-binding protein (I-FABP) is a cytosolic protein with a small molecular weight, 13-14 kDa.[11] It has a high degree of tissue specificity and accounts for approximately 2% of all the proteins in enterocytes at the tip of the intestinal villi.[12,13] It is thought to play a role in the intracellular utilization of fatty acids transport and metabolism.[14,15] During enterocyte necrosis, I-FABP is rapidly released into the circulatory system. Because of its small molecular weight, I-FABP has a relatively short half-life of 11 min in the serum, allowing it to easily pass through the glomerular apparatus and be collected in the urine.[16-18] For this reason, urine I-FABP has the potential for being an organ-specific biomarker for AMI.

We have previously shown in a preclinical model of AMI that I-FABP was a sensitive marker for intestinal ischemia.[19] We hypothesized that urine I-FABP will be an accurate biomarker in detecting intestinal ischemia in patients. In addition, we predict that surgical resection of the ischemic intestine will return I-FABP to normal levels.

Materials and methods

Patients

Patients who were clinically diagnosed with AMI and require surgical intervention were recruited to this study at the University of Alberta Hospital. This study was approved by the Human Research Ethics Board (Pro25429) at the University of Alberta, Edmonton. Informed consent was obtained from all study participants and privacy of all participants was strictly observed.

Inclusion and exclusion criteria

Patients who were over the age of 18 y, were suspected of having AMI, and had planned surgical intervention or endoscopy to obtain a gold standard diagnosis were included. To prevent confounders in biomarker interpretation patients who had other known intestinal pathologies, such as inflammatory bowel disease, celiac disease, and colorectal cancer were excluded from the study.

Biospecimen collection

Blood and urine were collected at three time points: preoperatively (within 1 hour before surgical intervention), 6 h and 24 h postoperatively. Collected blood was immediately spun and the separated serum was aliquoted and stored in −70°C until further use. The urine was collected at the same time points and sodium azide at 10% concentration was added, mixed well and subsequently spun at 5000 × g for 10 min at room temperature. Urine specimens were then aliquoted and also stored in −70°C until further use. Clinical information included age, sex, comorbidities, admission, discharge and pathologic diagnosis, clinical blood test, and surgical procedures were collected through chart review. Biospecimen analyses were done in a blinded manner since no information on diagnosis was known before analysis.

Sample preparation and enzyme-linked immunosorbent assay

Serum and urine specimens were analyzed using enzyme-linked immunosorbent assay (ELISA). I-FABP, interleukin-6 (IL-6), and tumor necrosis factor-α were measured using R&D Systems DuoSet (Minneapolis, MN). The protocol was followed according to the manufacturer. Briefly, serum samples were diluted 50% (vol/vol) in diluent from the R&D Systems kit. Urine samples were not diluted. The measurements from the ELISA experiments were made with SpectraMax M3 (Molecular Devices, Sunnyvale, CA). All samples were measured in duplicates, and background values were subtracted.