In Search for a Better Marker of Acute Pancreatitis—Third Time Lucky?

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Although several biomarkers of acute pancreatitis are currently defined, most are nonspecific, and their clinical performance has been disappointing. An article by Oiva et al. in the current issue of Clinical Chemistry describes the development of a specific assay for trypsinogen-3, a new biomarker that shows promise for the diagnosis of this condition (1). Acute pancreatitis accounts for >200,000 hospital admissions in the US every year (2). It carries a substantial mortality rate. Overall, about 20% of patients with acute pancreatitis have a severe course, and 10% to 30% of those with severe acute pancreatitis die. Despite the improvements in intensive care treatment during the past few decades, the death rate has not declined appreciably (3).

The clinical diagnosis of acute pancreatitis remains a challenge. Patients typically present with abdominal pain that is commonly associated with nausea and vomiting. These symptoms are nonspecific, however, and their onset depends on the underlying pathology (4). Not only do laboratory investigations augment the diagnosis of acute pancreatitis, they also form part of the diagnostic criteria and several scoring systems that determine the severity of the presentation. More than 100 years ago, the recommendation was to look for the presence of free fat and muscle fibers in the stools to assist in the diagnosis (5). The measurement of amylase and/or lipase, which are released from acinar cells, currently forms the cornerstone of routine diagnostic laboratory investigations (6). Of these assays, the relatively inexpensive serum amylase assay is the most widely used. Many conditions that might present with similar clinical symptoms are also associated with increased amylase concentrations. These conditions include cholecystitis, intestinal obstruction or ischemia, peptic ulcer disease, appendicitis, and gynecologic presentations. Serum amylase may also be increased in extra-abdominal states, such as pathologies of the salivary gland, impaired renal function, pneumonia, or such asymptomatic conditions as macroamylasemia (in which amylase is bound to immunoglobulins or polysaccharides to form large molecular weight complexes) (4, 7). Amylase concentrations >3 times the upper reference limit strongly support the diagnosis. Lower concentrations may be more difficult to interpret, because concentrations peak early and decline over 2 to 3 days and need to be explained in context with the onset of symptoms. Amylase concentrations may be falsely “normal” in cases of exocrine pancreatic insufficiency and in cases of analytical interference due to hypertriglyceridemia. Lipase activities parallel the increased activities seen with amylase and have the advantage of remaining increased for a longer time. Lipase activities also tend to decline to a lesser extent than for amylase in cases of chronic pancreatitis. One would therefore expect lipase to be more sensitive in late presentation and in states of acute or chronic pancreatitis. Despite this expectation, most studies have reported lipase and amylase to have similar specificities. Lipase is also known to increase in peptic ulcer disease, mesenteric ischemia, acute renal failure, bone fractures, crush injury, and fat embolism. In addition, analytical inaccuracies may be caused by the presence of multiple isoforms in the serum of pancreatitis patients and by the existence of macroforms (8). Alternative approaches, such as combined amylase and lipase testing, have been pursued to improve the diagnostic utility of amylase. This approach has not been widely adopted, however, because combined testing adds marginal, if any, diagnostic utility (9). Another important issue is assay standardization. Amylase and lipase are among the more poorly standardized tests in laboratory medicine. That might not be such an issue if their respective reference intervals were similar; however, some amylase assays produce values that are approximately 3 times those of other assays, and so misdiagnosing pancreatitis is a real possibility, especially if clinicians become accustomed to a single reference interval or if they work for several hospitals that have deployed different measurement platforms. Such clinical governance issues owing to a lack of assay standardization remain problematic, and laboratory medicine needs to address them in light of the huge advancements in other areas of medicine. Accordingly, the need remains for a more suitable test for diagnosing acute pancreatitis.
Trypsinogen is central to the pathogenesis of acute pancreatitis, which is caused by its unregulated activation to trypsin within pancreatic acinar cells. This process leads to autodigestion and inflammation. The pancreas produces 3 highly similar isoforms of trypsinogen (trypsinogen-1, –2, and –3), each of which is encoded by a different gene: PRSS1\(^3\) [protease, serine, 1 (trypsin 1)], PRSS2 [protease, serine, 2 (trypsin 2)], and PRSS3 [protease, serine, 3]. The trypsinogen genes are embedded among the genes of the T-cell receptor β locus (TRB\(^@\)) on chromosome 7. Their location raises the question as to whether their association arises from functional or regulatory constraints, or is inadvertent (10). This intercalation of the trypsinogen genes in the TRB\(^@\) locus is conserved in mice and chickens, suggesting shared functional or regulatory constraints. Interestingly, the PRSS3 gene encoding trypsinogen-3 has been translocated from chromosome 7q34 to chromosome 9p (10). Some of the trypsinogen genes are expressed in nonpancreatic tissues, where their function(s) are still unknown. In the brain, PRSS3 encodes a splicing variant that produces 2 trypsinogen forms (4A and 4B), the activated forms of which are identical to trypsin 3. Trypsins have been clearly implicated in cancer pathology (11).

The bulk of the trypsinogen secreted by the human pancreas consists of trypsinogen-1 and trypsinogen-2, with trypsinogen-3 making up <10% of the total. Trypsinogen-3 has some unique properties compared with trypsinogen-1 and trypsinogen-2. It has the ability to digest trypsin inhibitors, a property that raises the possibility that it may contribute to the pathogenesis of pancreatitis by reducing the protective concentrations of pancreatic secretory trypsin inhibitor. The other interesting property of trypsinogen-3 is its resistance to trypsin inhibitors (12). The fact that natural mutations of trypsin inhibitors are associated with chronic pancreatitis and familial pancreatitis indicates their important role in safeguarding against pancreatitis (13).

Specific assays for trypsinogen-1 and trypsinogen-2 were developed more than 2 decades ago (14). Of the assays for these 2 trypsinogens, the assay for urine trypsinogen-2 has gained the most interest (15), but mixed results have hampered its routine implementation (16). Now the same group, Oiva et al., report in this issue of Clinical Chemistry (1) on their use of various modern molecular biological techniques in developing a sandwich-type immunoassay for trypsinogen-3. In addition, they have produced a stable calibrator, an important and necessary requirement for a trypsinogen-3 assay. From 10 potential antibodies, they selected one that recognizes trypsinogen-3, trypsinogen-4B, and the activated form, trypsin-3 (but not trypsinogen-1 or –2). The implications of an antibody with such a specificity remains to be seen. The group determined the reference interval with a good-sized, well-characterized population of healthy individuals. The absence of any sex or age differences increases the simplicity of using this biomarker. The population distribution is unsurprisingly skewed to the right, with the median being lower than the 20% functional sensitivity and the highest concentration from the healthy population being approximately 3 times the 97.5th percentile. On the other hand, the area under the ROC curve was comparable to the areas under the curve for amylase and trypsinogen-2.

This study also revealed more on the etiologic and pathophysiologic differences of acute pancreatitis. Among patients with acute pancreatitis of various etiologies, trypsinogen-3 correlated with amylase only in patients with alcohol-induced disease. Trypsinogen-3 concentrations were significantly higher in alcohol-induced disease than in disease due to other etiologies. These findings and the potential relationship with carcinoma both warrant further studies.

Trypsinogen-3 is unique compared with trypsinogen-1 and trypsinogen-2, both with respect to its regulatory role and in the pathogenesis of acute pancreatitis. It seems to behave differently from other markers of acute pancreatitis, a feature that is likely related to the etiology. Determining whether this test is superior to amylase or lipase assays awaits testing in future studies. The cost of the assay will also influence its use in routine clinical practice. This encouraging report of Oiva et al. (1) should prompt further studies to elucidate the physiological role and the diagnostic and prognostic value of measuring trypsinogen-3 in acute pancreatitis. From a standardization viewpoint, let us hope that it will also be a case of third time lucky. Otherwise, to paraphrase Oscar Wilde, to fail a third time would not be unfortunate; it would be downright carelessness.

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\(^3\) Human genes: PRSS1, protease, serine, 1 (trypsin 1); PRSS2, protease, serine, 2 (trypsin 2); PRSS3, protease, serine, 3; TRB\(^@\), T-cell receptor β locus.
References


