Polyclonal pancreatic elastase assay is superior to monoclonal assay for diagnosis of acute pancreatitis

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We compared the clinical values for diagnosis of acute pancreatitis of two commercial assays for pancreatic elastase: an ELISA procedure with monoclonal antibodies and a RIA technique with polyclonal antibodies. In 14 patients with acute pancreatitis, serum concentrations of elastase determined by ELISA (ELISA-elastase) decreased much faster (half-life 0.4 days) than those of elastase determined by RIA (RIA-elastase) (2.2 days), amylase (0.8 days), or lipase (0.9 days). Serum samples from 253 additional patients with abdominal pain (32 of these with acute pancreatitis) were analyzed. In sera collected up to 48 h after the onset of disease, the ROC curves showed a slightly higher diagnostic value of RIA-elastase. In samples taken later, at a sensitivity of 90% the specificity of RIA-elastase was 95% (ELISA-elastase 40%). We conclude that serum ELISA-elastase is of much lower clinical value than RIA-elastase for diagnosis of acute pancreatitis.

In the past, measurement of numerous serum markers has been recommended for diagnosis of acute pancreatitis. Among these, nearly all pancreatic secretory enzymes such as amylase (EC 3.2.1.1), lipase (EC 3.1.1.3), trypsin (EC 3.4.21.4), elastase (EC 3.4.21.36), ribonuclease (EC 3.1.27.5), phospholipase A2 (EC 3.1.1.4), and carboxypeptidase B (EC 3.4.17.2) can be found [1–7]. Also, pancreatic proteins without enzymatic activity such as the pancreatitis-associated protein [8] or lithostathin [9] have been used for this purpose. For only a small number of markers, however, was sufficient data available to appreciate their value for diagnosis of pancreatic diseases [5]. In clinical practice, measurement of amylase or lipase is prevailing, and ROC curve analysis showed acceptable values for sensitivity and specificity [5].

Diagnosis of acute pancreatitis by measurement of serum enzymes was impaired when patients were admitted to the hospital several days after onset of symptoms. This was mostly due to the short half-life of the enzymes in serum [10]. Pancreatic elastase measured by polyclonal antibodies in a RIA system seems to be an exception, as this enzyme remained increased for >1 week after an attack of acute pancreatitis [11]. Therefore, measurement of elastase has been recommended in the past but not used widely, as the assay was only available as RIA. Recently, an ELISA technique with monoclonal antibodies was developed that might be used in smaller laboratories without availability of radioactive isotopes, or in emergency units. Our aim was to test and compare the clinical value of this new elastase ELISA with that of the RIA procedure.

Methods

Serum samples were taken daily from 14 patients with acute pancreatitis (ages 27–74 years, 10 men, 4 women; etiology: 6 biliary origin, 5 alcoholics, 3 unclear). The pancreatitis was verified by contrast-enhanced computed tomography (CT) or ultrasound.2 Commercially available kits from Boehringer Mannheim were used to measure enzyme activities of amylase (on the basis of hydrolysis of a p-nitrophenylmaltolheptaoside derivative, cat. no. 1 040 693) and lipase (turbidimetric measurement of triolein hydrolysis, cat. no. 1 268 449). For elastase assay, an ELISA with monoclonal antibodies (from Schebo Tech, cat. no. 06) and a RIA with polyclonal antibodies (from Abbott, cat. no. 1081–22) were used. For evaluation of diagnostic values the following upper limits of normal indicated by the manufacturers of the assays were used: amylase, 3.7 μmol/L; lipase, 3.1 μmol/L; polyclonal

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2 Nonstandard abbreviations: CT, computed tomography; A-2-MG, α2-macroglobulin; and A-1-AT, α1-antitrypsin.
elastase assay, 4 μg/L; monoclonal elastase assay, 3.5 μg/L.

An additional 253 individuals (ages 18–92 years, 147 men, 106 women) presenting with acute abdominal pain at our university hospitals and treated as inpatients were included into the study. The first serum sample was taken at admission but not later than 48 h (days 1–2) after onset of symptoms. Further samples were collected at 48–96 h (days 3–4) as well as at 96–144 h (days 5–6). Pancreatitis was diagnosed by CT or ultrasound. In the majority of patients (72%) ultrasound was used to exclude pancreatitis, whereas in only a minority of the nonpancreatitis group a CT was performed.

In 568 individuals without evidence of acute or chronic pancreatitis (normal amylase or lipase in serum, normal pancreas in ultrasound or CT), serum elastase was measured by the ELISA procedure to establish a reference range.

Levels of significance were calculated by a nonparametric sign test; at \( P < 0.05 \) a statistically significant difference was assumed. The ROC curves were calculated \cite{12} to evaluate the diagnostic value of the enzyme measurements. In accordance with the declaration of Helsinki of 1975 (revised in 1983), informed consent of the patients was obtained for use of their serum samples for scientific purposes.

**Results**

The precision of the elastase ELISA or RIA was controlled by repeated measurements of the same samples on different days and by multiple measurements of reference samples of low and high value on the same day. Precision of RIA and ELISA were similar; the RIA data were as follows: day-to-day reproducibility was 5.7% (at 1.5 μg/L) and 4.0% (CV) (at 15 μg/L); the CVs of samples measured on the same day were 3.9% (at 5 μg/L) and 5.7% (at 0.5 μg/L). The values were not influenced by repeated freezing and thawing (data not shown).

In all sera collected from the 14 pancreatitis patients on the first day of the disease, increased concentrations of elastase measured by RIA and by ELISA (RIA-elastase and ELISA-elastase, respectively), amylase, or lipase were found (Fig. 1A). After 3 days RIA-elastase was still increased in all patients, whereas in 11 of the 14 samples the values of ELISA-elastase were already within the normal range. After 1 week RIA-elastase was increased in all, but ELISA-elastase in none of the samples. On day 1 amylase, lipase, and RIA-elastase were threefold increased in all and ELISA-elastase in 12 of the 14 patients (Fig. 1B). RIA-elastase was threefold increased in half of the samples for >1 week. On day 2 ELISA-elastase was increased threefold above controls in 6 of 14 individuals and on day 3 in only one of 14 samples (Fig. 1B).

On the first day the serum concentrations of RIA- and ELISA-elastase were not different (Fig. 2A). But as already expected from the sensitivity data, the elastase concentration measured by ELISA decreased much faster and from the second day on both values were statistically different (Fig. 2A). The decreases of amylase and lipase in the course of the disease seemed to be parallel (Fig. 2A).

The enzyme concentrations in serum measured on the first 4 days were used to calculate, in each patient,
the respective half-lives. The individual values as well as the medians are shown in Fig. 2B. The half-life of RIA-elastase was 2.2 days, a value significantly higher ($P < 0.05$) than that of lipase (0.9 days) or amylase (0.8 days), and much higher ($P < 0.001$) than that of ELISA-elastase (0.4 days).

For further comparison of the clinical value of the two assay systems we performed elastase measurements in the serum of patients admitted to the clinic with acute abdominal pain. An acute pancreatitis was found in 32 of these individuals by CT or ultrasound. The ROC curves calculated from samples collected up to 48 h after onset of symptoms showed that the diagnostic quality of the RIA procedure was slightly higher than that of the ELISA technique (Fig. 3A): At a sensitivity of 95%, the specificity was near 95% (ELISA: 92%). With samples collected at 48–96 h the RIA showed much better results: At a sensitivity of 90% the specificity was 89% (ELISA-elastase <40%, Fig. 3B). The difference was even larger with samples at 96–144 h after onset of pancreatitis: At a sensitivity of 80%, the specificity of RIA-elastase was 80% and that of the ELISA-elastase <10% (Fig. 3C).

The diagnostic accuracy of elastase measurement [(true positives + true negatives)/all samples] was calculated in relation to different cutoff concentrations. It was a remarkable finding that the highest accuracy of ELISA-elastase was reached with a cutoff below the upper limit of normal (Fig. 4A), whereas the corresponding cutoff for RIA-elastase was at threefold normal (Fig. 4B). As anticipated from the ROC curves, the diagnostic accuracy of RIA-elastase was much higher than that of ELISA-elastase in samples collected later than 48 h.

To control the reference range of ELISA-elastase, in a series of 568 patients admitted to our gastroenterological clinic without evidence of pancreatitis (normal concentrations of pancreatic amylase and lipase, no evidence of acute pancreatitis in ultrasound or CT), concentrations of ELISA-elastase were measured in serum. Here we found that 95% of the values were <1.35 μg/L, 97% were <2.0 μg/L, and 99% were <2.2 μg/L.

Radioisotope assays for clinical diagnostics have been disappearing more or less from the panel of available techniques mainly because of increasingly complicated regulations for the handling of radionuclides. In accord with this, the pancreatic elastase assay with polyclonal antibodies in a RIA system has not been widely used, though the technique has been shown to have a high sensitivity [4]. By comparing the results of the recently available elastase ELISA with monoclonal antibodies with those of the RIA procedure with polyclonal antibodies, we found, surprisingly, that the latter assay with polyclonal antiserum was much better for diagnosis of pancreatitis than the ELISA system with monoclonals.

One major bias in this study may be our definition of pancreatitis. We chose to use as the sole indicator the findings in CT or ultrasound to assign patients to the pancreatitis group. It is generally assumed that by this procedure 10–15% of pancreatitis cases are overlooked, but precise data were not available. As we intended to compare the diagnostic efficiency of enzyme measurements, we had to use criteria independent of the results of serum enzymes. As an important advantage, by this procedure we completely avoided false positives in our pancreatitis group. On the other hand, pancreatitis must be excluded in all other patients. This cannot easily be achieved by any simple procedure, but in a recent publication sonography was known to exhibit a high negative predictive value of nearly 90% [13]. In this study, the prevalence of pancreatitis patients was even higher (28%) than in ours (12%). As in the majority of our patients, an ultrasound investigation was performed so we could be rather sure not to misclassify a significant number of patients.

Diagnostic accuracy and also ROC curves of RIA-elastase from samples collected at admission were previously analyzed in a series of papers [14–21]. They show, corresponding to our findings, a high sensitivity (>90%) at high specificity (>90%) of elastase, but also of other pancreatic secretory proteins. In these papers mostly
lipase was favored because of easy assay techniques. ELISA-elastase, however, has not been evaluated in detail; only a measurement of healthy blood donors has been performed [22]. We can show here that in samples collected early, RIA and ELISA measurements (i.e., measurements with polyclonal or monoclonal antibodies) were of rather similar clinical value, the RIA being slightly superior (Fig. 3A). However, the use of the cutoff value of 3.5 \( \mu \text{g/L} \) in the ELISA recommended by the manufacturer of the assay resulted in a much lower sensitivity. This, together with the accuracy data shown in Fig. 4, may suggest that the definition of the reference range of the ELISA may be a problem. Therefore ELISA-elastase measurements in 568 individuals without evidence of pancreatic diseases were done. In 97\% of the samples concentrations were <2 \( \mu \text{g/L} \). This was in contrast to the findings of others [22] in which 97\% of control sera concentrations were <3.5 \( \mu \text{g/L} \). Though this difference was obviously a small one, it may suggest the use of an upper limit of normal near 1.5–2 \( \mu \text{g/L} \). At this cutoff the sensitivity of the ELISA-elastase would be close to the RIA procedure, but only with samples collected up to 48 h.

The main diagnostic problem in pancreatitis, however, was that patients do not come to the clinic early after onset of pain. Individuals with chronic alcoholic pancreatitis, in particular, present late, so that the half-life of the enzymes in serum after an acute attack has to be taken into account [23–25]. This could lead to normal enzyme concentrations and, in turn, to overlooking acute pancreatitis in a significant number of patients [25]. When we focused on samples collected later after onset of pain we could show that RIA-elastase was persistently increased for \( >1 \) week. This was a finding similar to that reported by Büchler et al. [11]. ROC curves for the late samples have not been published yet; here we demonstrate that RIA-elastase was of high accuracy even a week after onset of pain, and at a sensitivity of 80\% the specificity was still near 80\%. The corresponding values for ELISA-elastase were far lower.

One reason for this large difference in clinical value could be the short half-life of ELISA-elastase (0.4 days) compared with the 2.2 days for RIA-elastase. At the moment we cannot offer any simple explanation for this difference. It is not very likely that this has anything to do with the type of the assay procedure, but seems to be associated with the antibodies used. In addition, elastase measurement in serum is difficult, as in its active form the enzyme is bound to \( \alpha_2 \)-macroglobulin (A-2-MG) and \( \alpha_1 \)-antitrypsin (A-1-AT). Whereas the complex with A-1-AT may be recognized by the polyclonal antibodies, the elastase bound to A-2-MG cannot be detected [26]. It has, however, not been determined whether this is also true for the monoclonal antibodies used in the ELISA. The situation may be even more complex during pancreatitis as both activated and nonactivated proelastase could be present in serum and recognized differently by the antibodies.
We could further speculate that monoclonal antibodies in the ELISA system mainly recognize the activated elastase, as antibodies were raised against the active form, and that the polyclonal antibodies in the RIA detect both activated and nonactivated forms. This could explain the high values immediately after onset of pancreatitis, where activated enzymes could be present in serum. After coupling of elastase to A-2-MG or A-1-AT the complex will be cleared at a higher rate from serum. In humans free A-2-MG had a half-life of >100 h, whereas its half-life was <10 min after formation of complexes with proteases [27].

Independent from the putative explanation for our findings, we conclude from the present data that only the polyclonal elastase assay can be recommended for diagnosis of acute pancreatitis in patients with abdominal pain. In particular, 2 days or more after onset of pain the sensitivity of the ELISA system was far too low.

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References


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