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ORIGINAL PAPER

Plasma Calprotectin Levels in Patients Suffering from Acute Pancreatitis

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Abstract Calprotectin (Cal) concentration is elevated in acute inflammatory reactions and its increase in the plasma suggests a diagnostic potential for Cal assay. This study aimed (a) to evaluate the Cal plasma levels in patients suffering from acute pancreatitis (AP) and (b) to assess whether early assay of Cal plasma levels can be helpful in assessment of the severity of AP. Forty-six consecutive patients, median age 45 years, suffering from a first attack of AP were recruited at two medical centers. Data collected on admission included age, sex, delay between pain onset and admission, and Glasgow score. A severe outcome was defined according to the Atlanta criteria. AP was defined as edematous or necrotic according to the CT findings. Plasma Cal and serum C reactive protein (CRP) were assayed in all patients within the first 24 hr after hospitalization. Sixty subjects suffering from blood hypertension were recruited as controls. Plasma Cal was measured by a commercial ELISA system. In all AP patients and in none of the controls, plasma Cal concentration was higher than the normal limit. Cal values in AP patients were significantly higher than in controls ($P < 0.0001$). There was not a statistically significant difference in Cal values between patients with severe and patients with mild AP. Plasma Cal values did not differ in necrotizing and edematous AP. During the follow-up plasma Cal was reassayed in six of the patients with abdominal fluid collection and the values were higher in the two patients with infected necrosis. We conclude that plasma Cal is elevated in patients with AP but it is not a useful marker for early prediction of pancreatitis severity. Further studies could evaluate its usefulness in pancreatic infected necrosis.

Keywords Calprotectin · Acute pancreatits · Pancreatic infected necrosis · Prognosis

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Introduction

Calprotectin (Cal) is a calcium binding protein belonging to the S-100 protein family and derived predominantly from neutrophils and monocytes [1, 2]. Various biological functions have been ascribed to Cal, including regulation in the inflammatory process [3] and antimicrobial activity [4]. Cal concentration is elevated in acute inflammatory reactions, and in some instances a correlation between Cal and other inflammation paramenters such as C reactive protein (CRP), erythrocyte sedimentation rate, and neutrophil granulocyte count has been shown [5]. As Cal is found in all tissues of the human body, its assessment in several fluids could distinguish between normal and pathological conditions [2]. For instance, an elevated Cal concentration in stools is considered a marker of intestinal neoplasias [6] or inflammatory bowel diseases [7] and helps to distinguish between organic causes of chronic diarrhea and irritable bowel syndrome [8].

An increased concentration of Cal in the plasma has been found in several organic diseases and suggests a diagnostic potential for Cal assay [2]. Despite these very promising data, no studies on the plasma Cal concentration in patients with acute pancreatits (AP) have been performed to date.

Aims of the present study were (a) to evaluate Cal plasma levels in patients suffering from AP and (b) to investigate whether early assay of Cal plasma levels could be helpful in assessment of the severity of AP.

Patients and methods

Forty-six consecutive patients (25 males), median age 45 years (range, 19–75 years), suffering from a first attack of AP were recruited at two medical centers, between January and December 2002. The diagnosis was based on the association of clinical symptoms, serum lipase and amylase increase, and characteristic computed tomographic (CT) findings. Patients with prior episodes of unexplained abdominal pain or with signs of chronic pancreatitis were excluded. Thirty-four patients had biliary AP, diagnosed on ultrasound or CT examinations; five had alcohol-induced AP, as they reported a daily intake of >100 g pure alcohol and an alcohol excess immediately before the acute attack; and in seven cases the etiology remained unknown (idiopathic AP). Data collected on admission included age, sex, delay from pain onset to admission, and Glasgow score [9]. A severe outcome was defined according to the Atlanta criteria [10]. AP was defined as edematous or necrotic according to the contrast-enhanced CT findings. Serum amylase and lipase and plasma Cal were assayed in all patients within the first 24 hr after hospitalisation; all the biochemical markers included in the Glasgow score and the CRP concentration were analyzed on the same blood sample.

Sixty subjects suffering from blood hypertension, sex and age matched with the study group, without inflammatory diseases or a history of acohol abuse, and with normal serum amylase values, were recruited as controls for plasma Cal values.

All subjects gave their informed consent to all procedures described in this study. The study protocol was approved by the Ethics Committee of the University Hospital of Palermo.

Plasma calprotectin determination

Blood samples were drawn into EDTA tubes and centrifuged at 2000 rpm for 15 min. Laboratory personnel unaware of the clinical diagnoses or details of the patients' clinical histories assayed plasma calprotectin. The concentrations were measured using a commercial ELISA system (Calprest; Eurospital, Trieste, Italy) based on polyclonal antibodies against calprotectin. Two 100-μl plasma aliquots from a single sample from each participant were assayed, and the mean of the two measurements was recorded. Threshold values supplied by the manufacturer (normal values, 0.3–1.6 μg/ml) were considered for statistical analysis. The intra-assay coefficient of variation (CV) for plasma Cal was 3.8% at a mean (SD) of 1.1 (0.2) ($n = 20$), and the interassay CV was 8%. The physicians who made the final evaluation of the AP severity were unaware of the Cal results throughout the study.

Statistical analysis

For the intergroup Cal concentration comparison, we used Mann-Whitney U test or Student's t test according to whether there was a non-Gaussian or Gaussian distribution of the data. The Spearman r correlation coefficient was used to correlate plasma Cal concentration with CRP and Glasgow score values.

Results

In all AP patients plasma Cal concentration was higher than the normal limit: the values ranged between 1.7 and 6.3 μg/ml. On the contary, none of the control subjects had values above the reference limit (Fig. 1). Plasma Cal values in AP patients were significantly higher than in controls: mean values (SD) were 5.3 (1.9) μg/ml in AP and 1.0 (0.3) μg/ml in controls ($P < 0.0001$). In patients with AP there was a trend toward higher plasma Cal values in cases with alcoholic etiology than in those due to gallstones (mean values $\pm$ SD, $5.6 + 1.2$ versus 5.1 ± 1.0 μg/ml; difference not statistically significant).

According to the Atlanta criteria for AP severity, 12 patients (26%) had severe AP and 34 (74%) had mild AP. Figure 2 shows the individual and median plasma Cal values in patients with severe and mild AP; there was a trend toward higher plasma Cal values in patients with severe pancreatitis, but the difference was not statistically significant in comparison with values observed in mild AP. Serum CRP concentration was higher in patients with severe AP than in those with a mild form, and this difference was statistically significant: mean (SD) values were 18.5 (4.3) mg/L in severe AP and 3.9 (2.9) mg/dl in mild forms ($P < 0.0001$). Also, the Glasgow score was significantly higher in patients with severe than in those with mild AP ($P = 0.04$). No statistically significant correlation was found between plasma Cal values and CRP concentration or Glasgow score.

Contrast-enhanced CT revealed necrotizing pancreatitis in 11 patients and edematous AP in the others; plasma Cal values, although higher in the subjects with necrosis, did not differ according to the presence or absence of the necrosis. Further CT evaluation showed the development of a

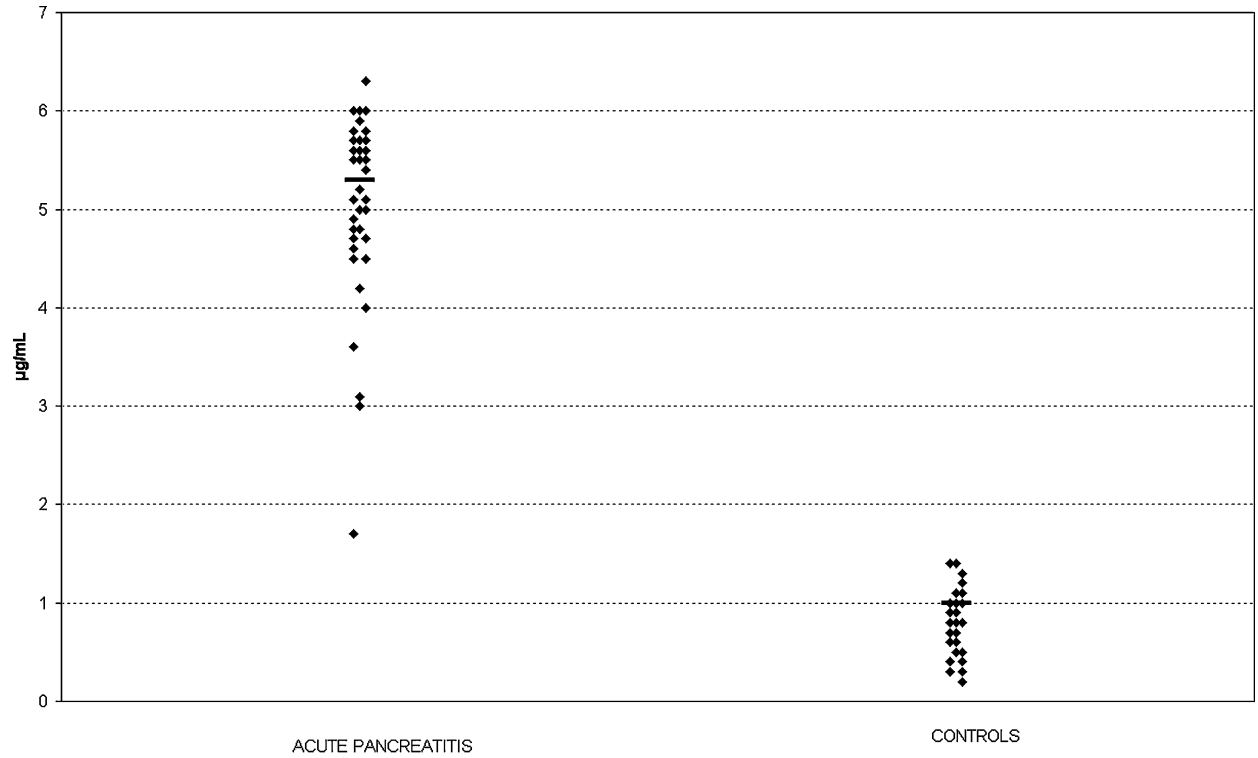

Fig. 1 Individual and mean (Barr) values of plasma calprotectin in patients with acute pancreatitis and in controls

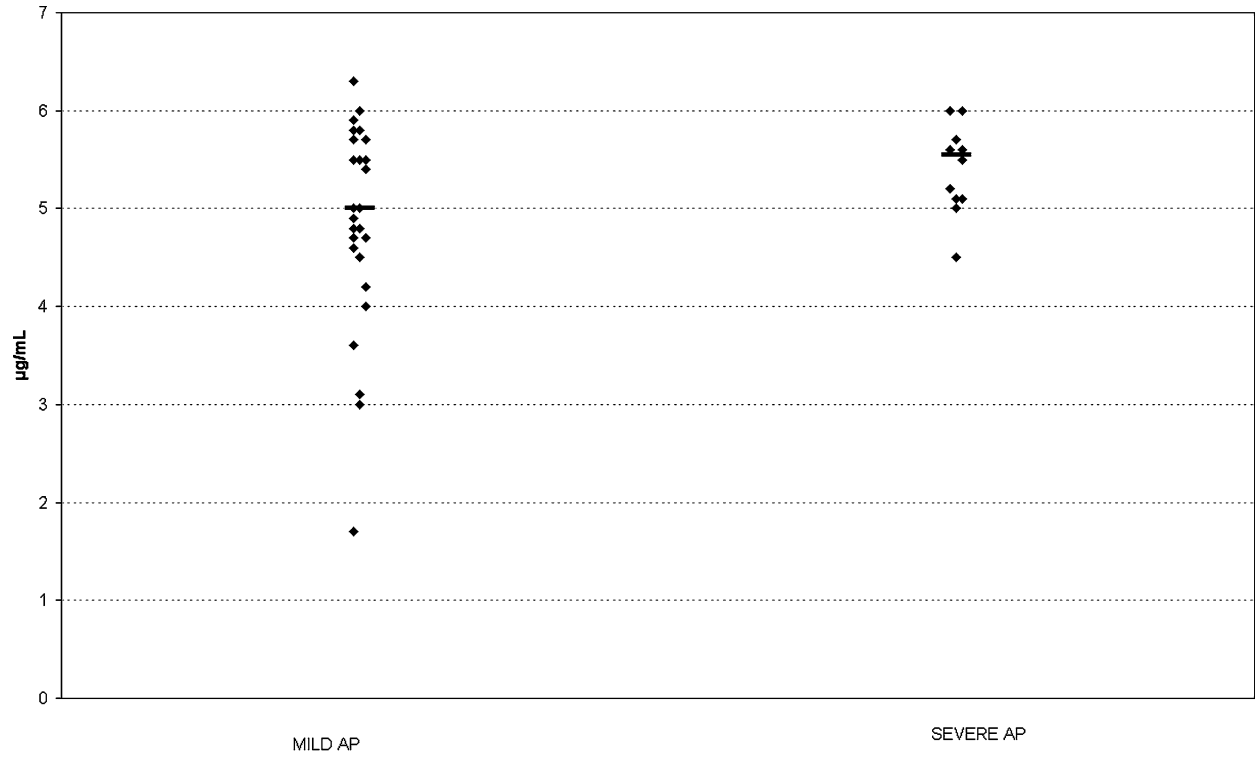

Fig. 2 Individual and mean (Barr) values of plasma calprotectin in patients with acute pancreatitis, divided according to the Atlanta criteria for severity of pancreatitis

pancreatic pseudocyst in five patients; plasma Cal values at hospitalisation did not differ between patients who developed a pseudocyst in comparison with all other AP patients.

During the hospital stay, pancreatic infected necrosis was found in two patients; at the moment of the hospitalization, the plasma Cal values of these two patients did not differ from those of all other AP patients. However, between the 6th and the10th days of hospitalization, plasma Cal was reassayed in six of the patients with abdominal fluid collection and suspected infected necrosis, including the two subjects who really had infected necrosis. The latter subjects had plasma Cal values higher than those of the others: 6.1 and 7.2 mg/dl in the patients with infected necrosis versus 3.2, 2.3, 1.8, and 1.8 mg/dl in the patients with fluid collection without infection.

Only two patients underwent surgical treatment, both for infected necrosis (necrosectomy with a left sided resection); one of them died in the first week after surgery.

Discussion

Plasma Cal is an interesting marker of inflammation. Previous studies demonstrated its usefulness in active rheumatic disease [5, 11], cystic fibrosis [12], pulmonary infections [13], and several other diseases [2]. In all cases the differences between normal and pathological levels suggested a diagnostic potential for Cal assay. Although Cal is mainly produced by neutrophils and monocytes, it is found in cells in all part of the body and its presence has also been demonstrated in pancreatic cells [14]. Despite these promising prospectives, no previous studies have evaluated the usefulnes of plasma Cal assay in patients with AP.

Our results demonstrate that plasma Cal is elevated in all patients with AP and in none of the controls; however, as we recruited control subjects without signs of acute inflammation, the high specificity of Cal in identifying AP patients cannot be considered a "real" result, and, on the other hand, serum amylase and lipase assays are considered sufficiently specific to confirm the clinical suspicion of AP. A more realistic goal in AP patients is the prediction of severity [15, 16]; in fact, early assessment of severity in AP can permit the selection of those patients who will benefit from a more intensive and early treatment with antibiotics [17] or antiprotease drugs [18], although this last point is still being debated [19, 20]. Unfortunately, in this respect, plasma Cal assay did not offer any advantage. Although there was a trend for higher Cal values in severe versus mild AP and in necrotizing versus edematous AP, the overlap of values between the groups did not permit any prediction of the course of the disease. On the contrary, serum CRP concentration confirmed its usefulness in distinguishing between severe and mild forms of AP [21, 22]. Obviously, it remains to verify whether a later assay of plasma Cal, or its monitoring during the hospital stay, could indicate the severity of AP, but even if so, a late prediction of AP severity is less important. However, the last result of the present study could be reevaluated in future researches: the use of plasma Cal as a marker of pancreatic infected necrosis. In fact, when plasma Cal was assayed in six patients with suspected infected necrosis, the highest values were recorded in the two patients who really suffered from infected necrosis.

In conclusion, we have shown that plasma Cal is elevated in patients with AP but it is not a useful marker for early prediction of the severity of pancreatitis. Further studies could evaluate its usefulness in patients who will develop infection of pancreatic necrosis, which still remains an unforeseeable event in the course of the disease.

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