Serum measurements of pancreatitis-associated protein in active Crohn’s disease with ileal location

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SUMMARY
Background and aims — Pancreatitis-associated protein (PAP) is a pancreatic stress protein also expressed in the ileum but not in the colon. Its serum concentration is increased in patients with small bowel inflammation due to untreated celiac disease. We searched to determine whether PAP could be a serum marker for ileal location of active Crohn’s disease (CD).

Methods — A multicenter prospective study was conducted, including 54 healthy controls and 124 patients with CD of whom 38 had quiescent ileal or ileocolonic disease (group A), 45 had active ileal or ileocolonic disease (group B), 18 had quiescent colon-only CD (group C), and 28 had active colonic disease (group D). Active disease was defined by a Crohn’s disease activity index > 150 and serum C-reactive protein (CRP) > 10 mg/mL. Location of lesions was assessed by endoscopy. PAP was assayed in serum, the upper threshold for normal values being 50 ng/mL.

Results — In group B, 27 patients (60%) had elevated serum PAP, compared to one in group A (2.5%), one in group C (5.3%), three in group D (10.7%) and none in the control group (P < 0.01). By contrast, serum levels of C-reactive protein did not differ between patients with active CD and either ileal location (group B) or pure colonic location (group D) (38 ± 10.5 vs 41.6 ± 6.4 mg/mL, NS). Within group B, serum PAP concentration was correlated with none of the epidemiological, clinical or biological data available. Increased serum level of PAP diagnosed ileal location in active CD with a sensitivity of 60%, a specificity of 94%, a positive predictive value of 84% and a negative predictive value of 81%.

Conclusion — Elevated serum PAP (> 50 ng/mL) is significantly associated with disease activity and ileal location.


The pathogenesis of Crohn’s disease (CD), which belongs to the group of inflammatory bowel diseases (IBD), remains unclear [1]. CD is a chronic disease characterized by recurrent acute relapses, most often unpredictable. Numerous studies have attempted to identify clinical or biological markers of acute relapse. Although clinical indexes of disease activity are available, such as the Harvey and Bradshaw or Van Hees’s indexes, the most widely used remains the Crohn’s disease activity index (CDAI) [2-4] which includes 18 clinical or biological parameters [2]. Among biological parameters, classical markers of inflammation such as the sedimentation test, leukocytosis and serum C-reactive protein are generally considered most reliable [5]. However, none of these indexes is specific of CD relapse or indicative of the location of CD lesions in the intestine. Intestinal alterations involve the small bowel, particularly the distal ileum, in 70% of CD patients. Among them, 30% present with exclusive small bowel location and 40% with a right ileocolonic location [6-8]. Only 25% of patients show lesions restricted to the colon, of whom 25% will eventually develop small bowel alterations [9].

Extension of CD lesions is not correlated to clinical activity, severity of mucosal damage evaluated by endoscopy or available biological markers [10]. While colonic lesions can be easily evidenced by total colonoscopy or rectosigmoidoscopy, assessing small bowel location requires small bowel barium radiology. Alternative isotopic procedures such as measurement of fecal excretion of 51CrCl3-labeled proteins or scintigraphy with 111Indium-labelled leucocytes are not routinely performed [11, 12]. Therefore, an easy procedure allowing detection of disease activity with small bowel location would be extremely useful in the follow-up of CD patients.

Pancreatitis-associated protein (PAP) was first described as a pancreatic secretory protein, not expressed under physiological condition in human or animals, but quickly and strongly overexpressed upon induction of pancreatic stress [13-15]. Serum levels of PAP correlate with the severity of acute pancreatitis [16]. More recently, PAP was also found expressed in rat and human intestinal epithelial cells, mainly in Paneth’s cells and in some caliciform cells [17-19]. PAP expression increases from the duodenum to the ileum, following the gradient of Paneth’s cell

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distribution along the gut, but is not expressed in the colon [17-19]. PAP mRNA levels are increased after small bowel mucosal injury in the mouse [18]. Serum levels of PAP are elevated in celiac disease patients and return to normal after a gluten-free diet has induced healing of intestinal mucosal atrophy [20]. Based on these findings, PAP serum levels might be elevated in CD patients with inflammatory activity of the small intestine. To test that hypothesis, we conducted a prospective study in which serum PAP was assayed in CD patients classified according to disease activity and location of lesions.

**Methods**

**Protocol**

A multicenter prospective controlled study involving the French University Hospitals of Marseille, Nice and Rennes was conducted between January 1998 and April 1999. Ethical committee agreement was obtained from the Centre d'Investigations Cliniques of Marseille. Patients or controls with previously known acute or chronic pancreatitis, colorectal carcinoma, intestinal disease with mucosal injury, previous intestinal resection or chronic renal insufficiency were excluded. Patients and controls were submitted to a standardized questionnaire. Data included age, sex, tobacco consumption, familial history of CD, previous colonic or pancreatic disease. In CD patients, data also included duration of the disease, intestinal location, CD activity and presence of extraintestinal locations. Therapeutic data (drugs, surgery, nutritional therapy) were recorded. Clinical information on CD obtained from patients included duration of the disease, location, CD activity, extraintestinal location, and previous therapy (drugs, surgery, nutritional therapy). The intestinal location of the CD was based on endoscopic investigations possibly associated with small bowel barium meal performed within a period no longer than one year prior to the patient inclusion in the study. When more detailed information concerning the history of CD was required, the medical referree was contacted with the patient’s agreement. For each patient the CDAI was calculated [2]. Serum samples were obtained at inclusion in controls and patients. Serum levels of C-reactive protein, fibrin, gamma glutamyl transferase, alkaline phosphatase, liver transaminases, amylase, lipase, creatinine were measured. Serum samples for PAP assays were obtained from 5 mL blood and stored at −70°C until use. PAP was assayed using a commercial ELISA test (PancRePAP, Dynabio SA, Marseille). The threshold for PAP normal values was set to 50 ng/mL as determined from previous studies [20]. CD was considered active in patients with a CDAI > 150 and C-reactive protein > 10 mg/mL in order to rule out the patients presenting with a "false" increased CDAI, as it can occur in case of fibrotic stenosis or extended ileal resection.

**Patients**

One hundred and forty patients and 60 controls were enrolled in this prospective study. Seventeen out of the initial 200 subjects were excluded because 4 presented with pancreatitis at admission or during the following 30 days, one had a false diagnosis of CD and a final diagnosis of ulcerative colitis and, for the 12 remaining subjects, PAP assays could not be performed because of hemolysis. Finally, complete data from 129 patients and 54 controls with adequate inclusion criteria were available. The 129 patients, 59 men and 70 women, had a mean age of 34 years (18–62 years). The 129 patients were classified into 4 groups according to disease activity and location of lesions: group A, ileal or ileocolonic location (without inflammation activity); group B, ileal or ileocolonic location with inflammatory activity (45 patients); group C, pure colonic location without inflammatory activity (18 patients); group D, pure colonic location with inflammatory activity (28 patients).

**Data analysis**

Data from each patient were recorded on a standardized questionnaire. Data were entered in a database software (Excel 5.0, Microsoft, USA), then transferred to a statistical software (Statview 4.5, USA). For comparison between groups, qualitative data were analyzed by the χ² test with Yate’s corrections if necessary and by the Fisher’s exact test for 2 × 2 contingency tables. Quantitative data were analyzed with the Student’s t test for the comparison of 2 samples. Simultaneous comparisons of more than two samples were performed using an ANOVA test in case of normal distribution and the Kruskal-Wallis (non parametric) test otherwise. Linear parametric correlations were performed with the r² test. The statistical significance threshold was set at P = 0.05. Results were expressed as mean values ± standard deviation (SD). ROC curves were performed to measure the accuracy of PAP thresholds in assessing the diagnosis of ileal involvement with active inflammatory CD (SAS Software, USA).

**Results**

**Crohn’s disease features**

The average age at onset of the disease was 27 years (± 11). The mean duration of CD at inclusion was 7 years (± 6). Fourteen patients (11%) had familial cases of CD. Forty patients (31%) had appendectomy and 26 patients (20%) had tonsillectomy. Previous surgery of CD patients involved the small bowel (n = 32), the colorectum (n = 31) and the perianal region (n = 23). At inclusion, 35 patients were taking immunosuppressive drugs, 54 corticosteroids, 24 antibiotics, 61 aminosalicylates and 18 were receiving artificial nutrition. Extraintestinal locations of CD, mainly arthropathy, were found in 31 cases (24%). No patient had duodenal involvement of CD. Lesions were located in the ileum in 64% of the patients (groups A and B) and restricted to the colon in 35% of the cases (groups C and D). CD was active in 73 patients (56%, groups B and D).

**Clinical and epidemiological data**

The 54 controls had a mean age of 49 ± 11 years, significantly different from that of the 129 patients, mean age 34 ± 12.8 (P < 0.001). Their average weight was also significantly higher (66 ± 13 vs 59 ± 12, P = 0.002). The percentage of smokers was significantly lower in controls (20% vs 45.8%, P < 0.03). The M/F sex ratio was similar in controls (0.80) and patients (0.84).

Comparisons were also made among groups of patients. In groups A, B, C and D, the average age (years) was 32 ± 13, 31 ± 10, 41 ± 17 and 35 ± 11, respectively (P = 0.04). The M/F sex ratio was 43%, 64%, 68% and 57% (NS). The percentage of teetotallers was 8%, 7%, 21% and 7% (NS) and the mean body weight was 60 kg, 57 kg, 61 kg and 61 kg (NS). However, the percentage of smokers, 46%, 63%, 16% and 36%, differed among groups (P = 0.03).

**PAP serum values**

Serum levels of PAP in the four groups of patients are given in table I. Differences between mean values in groups A, B, C, D and control were statistically significant (P < 0.01). Figure 1 shows the distribution of PAP values in the 4 groups. In group B, PAP was above the 50 ng/mL threshold in 27 of the 45 cases (60%), compared to 1/38 (2.5%) in group A, 1/18 (5.3%) in group C and 3/28 (10.7%) in group D (table I and figure 1) (P < 0.01). No PAP value exceeded the threshold in the control group, with a maximal value of 48 ng/mL. Based on these data, a PAP serum concentration above 50 ng/mL would diagnose active CD with ileal location with a sensitivity of 60% and a specificity of 94%. The positive predictive value would be 84% and the negative predictive value would be 81%. All PAP
thresholds are shown in Table II and ROC curves in Figure 2. By contrast, serum levels of C-reactive protein did not differ between patients with active CD and either ileal location (group B) or pure colonic location (group D) (38 ±10.5 vs. 41.6 ±6.4 mg/mL, NS). A small positive linear correlation between age and serum level of PAP was found in controls (r² = 0.30; P = 0.02).

Epidemiological, clinical and therapeutical parameters in group B patients with normal or elevated PAP values

Patients from group B (active disease and ileal location) were classified into two subclasses: those with normal PAP (18/45, 40%) and those with increased values (27/45, 60%). Epidemiological, clinical or therapeutical data from patients in the two subclasses were compared: sex ratio (55 vs 61%), smokers (60% vs 88%), teetotallers (22% vs 22%), age (25 vs 24 years), duration of CD (7.3 vs 6.6 years), weight loss (4.8 vs 4.5 kg), CDAI (241 vs 284), extraintestinal location (74% vs 61%), familial cases (12% vs 27%), appendicectomy (26% vs 44%), tonsillectomy (18% vs 33%), previous ileal surgery (30% vs 23%) and serum C-reactive protein (46.2 vs 34.6 mg/mL) were not significantly different. No difference was observed in therapeutic management, including intake of immunosuppressive drugs (33% vs 27%), corticosteroids (48% vs 44%), antibiotics (37% vs 16%), aminosalicylates (37% vs 38%) and artificial nutrition (33% vs 27%) between group B patients with and without normal PAP levels, respectively.

| Group A : Ileal, no inflammation (n = 38) | 33.1 ± 2.5 | 15-64 | 1 (2.5 %) |
| Group B : Ileal, inflammation (n = 45)    | 65.3 ± 38.8 * | 17-410 | 27 b (60 %) |
| Group C : Colonic, no inflammation (n = 18) | 29.5 ± 3 | 10-52 | 1 (5.3 %) |
| Group D : Colonic, inflammation (n = 28)  | 34.4 ± 4.8 | 13-60 | 3 (10.7 %) |
| Controls (n = 54)                          | 27.4 ± 1.3 | 12-46 | 0 (0 %) |

* Significantly different from values in other groups (P<0.01). b Significantly different from values in patient groups (P<0.01).

Table II. – Variation of sensitivity and specificity of PAP thresholds in ROC curve.

<table>
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<tr>
<th>PAP thresholds (ng/mL)</th>
<th>Sensitivity</th>
<th>Specificity</th>
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<td>11.6</td>
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<td>0.007</td>
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<tr>
<td>20.49</td>
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<td>0.016</td>
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<td>40.00</td>
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<td>45.08</td>
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<td>50.00</td>
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<td>101.97</td>
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</table>
Discussion

CDAI and C-reactive protein are widely used for monitoring the activity of CD but do not provide information on location or extension of the lesions [2, 5, 10]. Endoscopy or small bowel barium meal cannot be easily repeated during the course of CD. New tests providing additional information, easily and at low cost, would therefore be welcome. The diagnostic value of serum PAP concentration in intestinal inflammatory diseases was suggested by a study on patients with celiac disease showing elevated serum PAP during inflammation and the return to normal values upon control of the disease by a gluten-free diet [20]. Because celiac disease affects the small intestine and PAP is expressed in the small intestine mucosa, but not in the colon [17-19], it was conceivable that serum PAP would also be increased in active Crohn’s disease with ileal location.

That hypothesis was tested in a prospective multicenter study. CD patients enrolled in the study were classified into 4 groups, according to the quiescent or active status of the disease and the localization, purely colonic or ileo-colonic, of the lesions. The intestinal localization of the CD was assessed, less than one year before the inclusion of the patient in the study, by colonoscopy and ileoscopy and in some patients by small bowel barium meal. Because it was a pilot study and for ethical considerations, endoscopic or radiological investigations were only performed at inclusion if they were required for the therapeutic management.

The control group was composed of volunteers with irritable bowel syndrome and without pancreatic problems. When comparing serum PAP values in patients and controls, only patients with active disease and ileal location had a significant elevation (65.3 ± 38.8 vs 27.4 ± 1.3 ng/mL), supporting the hypothesis that inflammation of the ileum, but not that of the colon, results in increased serum PAP (Table I).

To evaluate the clinical significance of these results, it was important to check that the studied groups could be adequately compared. Patients and controls had similar sex ratio, but differed in tobacco consumption and mean age. Tobacco consumption, which is a risk factor for CD, was more frequent in patients, as expected from previous studies [21, 22]. However, there was no significant difference in serum PAP values between controls smokers and controls non-smokers, suggesting that increased tobacco consumption was not responsible for PAP elevation in the patients. Also, controls were older than patients (mean age 49 yrs vs 34 yrs). CD usually affects young adults. In a study conducted in Minnesota from 1940 to 1993, the peak age incidence was 29 years [9], a value close to that observed in the present study. Better adjustment for age of the control group was impossible because inclusion required previous colonoscopy with normal results, and indications for such procedure are infrequent in young people. To check whether the difference in mean age between patients and controls could induce a bias, we looked for a correlation between age and serum PAP level in controls. A small but significant positive linear correlation was indeed found (r² = 0.30; P = 0.02). However, adjustment for age would decrease PAP control values and slightly increase the observed differences with patients.

The observation that serum PAP levels are significantly increased in CD patients with active disease and ileal location may be interesting in clinical practice. Previous determinations of serum PAP in the normal population have shown mean values ranging from 22 to 27.7 ng/mL, with less than 2% of values exceeding 50 ng/mL [20, 23]. In our control group, the average PAP concentration was 27.4 ± 1.3 ng/mL, the highest value being 48 ng/mL. The upper threshold of the normal values was therefore arbitrarily set to 50 ng/mL. Sixty percent of cases with ileal location and active disease were above that threshold, compared to 2.5% in quiescent CD with similar location, and 10.7% or 5.3% in patients with pure colonic location in active or quiescent CD, respectively (Table I). Therefore, a serum PAP concentration above 50 ng/mL is associated with ileal active CD with a 60% sensitivity and 94% specificity, the positive and negative predictive values being 84% and 81%, respectively. Different PAP thresholds with their related sensitivity and specificity are shown in Table I and Figure 2. Sensitivity and specificity are increasing or decreasing in opposite distribution with respectively values of 0.95 and 0.16 at 20.49 ng/mL and 0.43 and 0.97 at 60.35 ng/mL (Table II).

Figure 1 shows that 40% of patients in group B had serum PAP levels within the normal range, suggesting that these patients differed in some way from patients with elevated PAP levels. Clinical and epidemiological data of these patients and patients from group B with elevated PAP levels were compared: no differences could be observed, nor in their previous medical or surgical management. Thus, available data could not account for differences in serum PAP levels among patients with active ileal CD. A possible explanation could be that their profile of cytokine synthesis, known to influence PAP expression [1, 24-27], was different. Alternatively, serum PAP and serum pancreatic amylase and lipase levels, as markers of pancreatitis and increased serum PAP, as evidenced during acute pancreatitis [19]. Furthermore, the existence of IBD-associated pancreatitis was recently demonstrated [30]. Patients with history of chronic or acute pancreatitis were excluded from this study. Three patients presenting at admission with clinical signs of pancreatitis and elevated serum amylase and lipase levels and a fourth patient with normal serum amylase and lipase levels but with a PAP level of 1609 ng/mL because of drug-induced pancreatitis (azathioprine), were eventually excluded. The fact that 4 CD patients among 140 (2.8%) had pancreatitis is in the range of previous reports [30]. However, IBD-associated pancreatitis is often a silent disease, as shown by autopsy studies reporting pancreatic fibrosis in 38% of CD patients without pancreatic symptoms [31]. Therefore, in some patients from our study, pancreatic alteration may have contributed to serum PAP elevation, but that influence should be limited because all patients had normal serum amylase and lipase levels. In addition, this should not affect the conclusions of this work: since previous studies did not show any relationship between pancreatitis and disease activity or location of the lesions [30], the potential pancreatic involvement should be equally distributed within the 4 groups.

In conclusion, a serum PAP concentration higher than 50 ng/mL is significantly associated with ileal location in active CD, with 60% sensitivity and 94% specificity. Despite a sensitivity which should be improved, PAP is to our knowledge the first
biological marker that provides information on the localisation of CD. Further studies are required to understand why ileal inflammation is associated with elevated PAP in only 60% of the cases and the possibility that PAP levels reflect the extension of ileal lesions should be investigated. Whether or not PAP could be an indicator for the therapeutic management of CD might be assessed in a further longitudinal prospective study.

**Références**


