Factors contributing to infectious diarrhea-associated pancreatic enzyme alterations

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SUMMARY

Objectives — Several pathogens have been involved as etiologic agents of acute pancreatitis. We studied 59 patients presenting acute infectious diarrhea in order to determine the incidence as well as to identify factors which may contribute to the occurrence of pancreatic enzyme alteration or true acute pancreatitis.

Methods — Patients were evaluated for serum lipase and amylase, and 24-hours urinary amylase. Clinical and biological parameters were noted. Abdominal sonography and rectosigmoidoscopy were performed.

Results — Pancreatic enzyme alteration was found in 24% of patients. Twelve had salmonellosis and 2 Campylobacter jejuni infection. They had more prolonged diarrhea, more frequent renal impairment and increased triglyceridemia. Triglyceridemia was correlated to blood amylase, inflammatory syndrome and renal impairment. Serum amylase was linked to serum urea and creatinine and to biological markers of inflammation. Three patients had true acute pancreatitis.

Conclusion — Patients presenting dysentery-like infectious diarrhea and upper abdominal pain should be investigated for concomitant pancreatic reaction or acute pancreatitis which seems more frequent in patients with enterocolitis due to enteroinvasive microbes, mostly non-typhoidal Salmonella. Pancreatic disturbances are related to the severity of these infections. However, overt infectious diarrhea-associated pancreatitis is a rare event.

Introduction

Cholelithiasis and alcoholism are the most frequent causes of acute pancreatitis. Together, they account for 80 to 90% of patients given the diagnosis of acute pancreatitis. Other pathologic conditions including metabolic disturbances (hypercalcemia, hyperlipidemia), drugs, abdominal trauma or abdominal surgery, may be recognized as the cause of the disease. Occasionally, microbial pathogens have been suggested to induce acute non-alcoholic, non-biliary pancreatitis. Numerous case reports described association of a wide variety of infectious agents [1], including viruses such as mump-virus or coxsackie-B virus [2, 3], bacteria (Leptospira species [4, 5], Campylobacter jejuni [6, 7], Mycoplasma pneumoniae [8], Yersinia species [9], Brucella bacteria [10, 11], Legionella strains [12], Mycobacteria tuberculosis [13], Salmonella typhi [14-17] or non-typhoidal Salmonella strains [18-28]), and parasites (Ascaris lumbricoides or Clonorchis sinensis), to acute pancreatitis.

RéSUMÉ

Facteurs associés aux anomalies pancréatiques compliquant les diarrhées infectieuses

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Méthodes — L’activité des amylases et lipases sérées, des amylases urinaires 24 heures, ainsi que certains paramètres cliniques et biologiques (statut inflammatoire, fonction rénale,…) ont été évalués au moment de l’admission pour diarrhée infectieuse aiguë. Une échographie abdominale et une rectosigmoidoscopie étaient également réalisées.


Conclusion — Chez les malades présentant une diarrhée infectieuse aiguë dysentérique et des douleurs abdominales épigastriques prédominantes, une réaction biologique pancréatique voire plus rarement une pancréatite aiguë, doivent être recherchées. Ces anomalies apparaissent plus fréquentes avec des germes entéroinvasifs, en particulier lors des salmonelloses non-typhiques. Elles semblent liées à la sévérité de l’agression et de la réponse inflammatoire qui en résulte. La survenue d’une pancréatite aiguë authentique reste néanmoins un événement rare.
In the present study we prospectively assessed the frequency of pancreatic enzymes alterations or acute pancreatitis in 59 consecutive patients hospitalized for acute severe infectious enteritis and/or colitis, and tried to identify several factors which could contribute to their occurrence.

## Methods

### Patients

Fifty-nine consecutive patients (37 women and 22 men, median age: 38 years, range: 15-94) admitted for acute infectious diarrhea were prospectively included in the study. All patients presented with dysenteric-like (sometimes bloody, N = 12, 20.3%) diarrhea rather than watery diarrhea. Microbiological diagnosis is summarized in table I. Alteration in pancreatic enzymes activity was defined by an increase in serum lipase and at least a threefold increase of serum or 24-hours urinary amylase, followed by normalization of pancreatic enzymes concentrations. Acute pancreatitis has been defined as an acute inflammation of the pancreas characterised by typical abdominal pain and associated with an increase of pancreatic enzymes in serum and/or urine and confirmed by abnormalities of the gland at imaging [29].

### Clinical assessment

The following clinical parameters were recorded: number of bowel movements per day, duration of diarrhea, subjective intensity of abdominal pain (none = 0, mild = 1, moderate = 2, severe = 3), presence of extra-intestinal symptoms (arthritis, erythema nodosum) and length of hospital stay. Patients were asked for personal and familial history of biliary disease, endocrine disorders and alcohol habits.

Rectosigmoidoscopy and abdominal ultrasonography [30] were performed in all patients.

### Biological parameters

Samples were collected at admission using standard procedures. They included serum lipase (normal values < 200 IU/L) and amylase (normal values < 53 IU/L) activities, 24-hours urinary amylase (normal values < 250 IU/24 hours) activity, concentrations of inflammatory markers: erythrocyte sedimentation rate (ESR), fibrinogen, C-reactive protein (CRP), albumin, as well as measurement of plasma neopterin (as a marker of monocyte/macrophage activation, N = 15) and soluble interleukin-2 receptors (sIL2R, N = 13) as a marker of lymphocyte activation.

Furthermore, in 21 patients serum samples were kept at -80°C in order to determine systemic tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and IL-6 concentrations using ELISA (Antibody Solutions, Half Moon Bay, CA, USA). Serum ALT and AST, alkaline phosphatase, γ-glutamyltransferase and total cholesterol levels, and finally serum calcium concentration were measured in order to exclude other potential etiologies (i.e. alcoholic, biliary or metabolic) for pancreatic reaction or acute pancreatitis.

In all patients, blood culture (at admission and in case of recrudescence of septic signs thereafter) as well as stool culture (at admission) were performed using standard techniques.

### Statistics

Data are expressed as median (range). Statistical differences between groups were evaluated by Student's t-test. Correlations were calculated using linear regression (r), or the non-parametric Spearman rank test methods (r′, when N < 30). Significance was set at P < 0.05.

## Results

### Pancreatic reaction

An increase in serum lipase, and serum and/or 24-hours urinary amylase concentrations was observed in 14/59 (24%) patients. Among them 12 (85.7%) presented diarrhea related to salmonellosa infection: 11 non-typhoidal salmonellosis (S. bovis: N = 1, S. braenderup: N = 1, S. enteritidis: N = 6, S. heidelberg: N = 1, S. typhimurium: N = 2), and one S. typhi. In 2 patients infectious diarrhea was caused by Campylobacter jejuni. This group of patients was considered as having an altered pancreatic reaction (PR), and therefore compared to the group of patients without pancreatic disturbances [called non pancreatic reaction group (NPR), N = 45]. No patient in either group had positive blood culture. In the PR patients, median serum lipase activity was 473 (141-2490) IU/L (versus 35 (11-260) IU/L in NPR, P < 0.0001), median serum amylase activity 150 (range: 13-428) IU/L (33 (15-98) IU/L in NPR, P < 0.0001) and 24-hours urinary amylase activity 1853 (299-9720) IU/L (NPR = 219 (32-1500) IU/L, P < 0.001 (table II)). Pancreatic enzymes were positively linked to each other. Considering clinical parameters no patient presented with septic shock. Length of hospital stay was slightly but not significantly increased in PR patients (median: 9, range: 4-19) compared to NPR patients (median: 7, range: 1-19; P = 0.15). Two PR patients (14%) presented extraintestinal manifestations (arthritis alone: N = 1, arthritis and erythema nodosum: N = 1) compared to 4/45 (9%; arthritis) in the NPR group (P = 0.57). Median frequency of bowel movements did not differ between PR and NPR patients [PR: 7 (3-15), NPR: 7 (3-20); P = 0.63], but duration of diarrhea and subjective assessment of abdominal pain (typically epigastric pain) were significantly higher in PR patients compared to patients without increase in pancreatic enzymes [median: 6 (4-15) versus median: 4 (2-28) days, P < 0.05 and 2 (0-3) versus 1 (0-2), P < 0.01 respectively]. In the former group of patients, plasma triglyceride concentration was higher (P < 0.01) and renal impairment more common with a statistically significant increase in urea (P < 0.0001, table II) and creatinine (P < 0.001). Furthermore, biological parameters of inflammatory and immune activation were higher in patients presenting PR as observed for ESR (P < 0.001), fibrinogen (P < 0.01), blood neopterin (P < 0.05) and sIL2R (P < 0.05). In the PR group, 3 patients had increased cytokine plasma concentrations (none in the NPR group).
group). However, usually, cytokine concentrations were below the detection limit in both groups explaining the absence of statistical differences between PR and NPR patients concerning TNF-α, IL-1β or IL-6 concentrations.

Among patients presenting pancreatic reaction, only 3/14 (21.4%) exhibited signs of mild acute pancreatitis at abdominal ultrasonography, usually pancreatic swelling regressive with clinical and biological improvement. Only the patient presenting Salmonella typhi-associated pancreatitis had limited pancreatic necrosis attested by computed tomography. In 53/59 (90%) of patients, endoscopic features (segmental or diffuse mucosal redness, superficial or deep ulcerations) compatible with acute infectious colitis were present without difference between PR and NPR patients.

### Correlation between triglyceride concentration and other parameters

Plasma triglyceride concentration was positively linked to serum amylase activity \((r = 0.45, P < 0.001, \text{linear regression}; \text{figure 1a})\), urea \((r = 0.52, P < 0.0001, \text{linear regression})\) and creatinine \((r = 0.58, P < 0.0001, \text{linear regression}; \text{figure 1b})\) concentrations, ESR \((r' = 0.45, P < 0.01, \text{Spearman rank test})\), fibrinogen \((r' = 0.34, P < 0.05, \text{Spearman rank test})\), blood neopterin \((r' = 0.9, P < 0.01, \text{Spearman rank test}; \text{figure 1c})\), sRIL2 \((r' = 0.64, P < 0.05, \text{Spearman rank test})\) and IL-6 \((r' = 0.63, P < 0.01, \text{Spearman rank test})\) concentrations.

### Correlation between markers of renal function and other parameters

Urea and serum creatinine were positively correlated to serum amylase activity \((r = 0.44, P < 0.001\) and \(r = 0.45, P < 0.001\) respectively, linear regression). They were also positively linked to TNF-α \((r' = 0.54, P < 0.05\) and \(r' = 0.53, P < 0.05\) respectively, Spearman rank test), to IL-6 \((r' = 0.62, P < 0.001\) and \(r = 0.6, P < 0.01\) respectively, Spearman rank test), and, creatinine to IL-1β \((r' = 0.45, P < 0.05, \text{Spearman rank test})\).

### Correlation between inflammatory and immune activation and pancreatic reaction

Serum amylase activity was positively linked to ESR \((r = 0.43, P < 0.01, \text{linear regression})\), TNF-α \((r' = 0.53, P < 0.05, \text{Spearman rank test})\), IL-6 \((r' = 0.59, P < 0.01, \text{Spearman rank test})\), and sRIL2 \((r' = 0.57, P < 0.05, \text{Spearman rank test})\). 24-hours urinary amylase activity was positively linked to ESR \((r = 0.54, P < 0.001, \text{linear regression})\) and to TNF-α \((r' = 0.5, P < 0.05, \text{Spearman rank test})\). There was a trend to a positive link between 24-hours urinary amylase activity and IL-1β on one hand \((r' = 0.44, P = 0.059, \text{Spearman rank test})\), and IL-6 on the other hand \((r' = 0.44, P = 0.061, \text{Spearman rank test})\). Serum lipase activity was only linked to sRIL2 \((r' = 0.72, P < 0.05, \text{Spearman rank test})\) despite a trend to a positive relation with TNF-α \((r' = 0.45, P = 0.08)\) and IL-6 \((r' = 0.43, P = 0.09)\).

### Discussion

The frequency and the pathophysiology of non-typhoidal Salmonella-associated pancreatic reaction or acute pancreatitis still remain a matter of debate. Renner et al. [18] in an 18-month prospective study included 47 cases of Salmonella enterocolitis for clinical and laboratory features of associated pancreatic reaction or acute pancreatitis. They found concomitant pancreatitis in 7/16 (44%) patients presenting Salmonella typhimurium infection and in 22/31 (71%) with Salmonella enteritidis-related diarrhea. By contrast, Murphy et al. [19] found no elevated serum amylase in a retrospective analysis of 51 patients with non-typhoidal Salmonella enteritis; Tositti et al. reported the presence of hyperamylasemia in only 10.2% of 507 consecutive patients with acute gastroenteritis [27], and recently, Pezzilli et al. [28] reported an increase in serum amylase and serum lipase respectively in 6.7% and 16.7% among 30 patients presenting with salmonellosis (S. enteritidis in 25 patients and S. typhimurium in 5).

We prospectively studied the occurrence of alteration in pancreatic enzymes activity in patients presenting acute infectious diarrhea requiring hospitalization, and investigated for potential...
study to the other, and presumably by referral biases. Actually, considering patients with radiological signs of pancreatitis (defined as patients having true acute pancreatitis [29]), in our series, only 3 patients could be regarded as having true acute pancreatitis (21.4% of those presenting marked biological signs of pancreatic enzyme alterations, 5.1% of all patients), the remainder having what we called pancreatic reaction. However, if only increase in serum amylase and/or urinary amylase (including levels below the limits we considered as relevant) without the need of having increased serum lipase was considered as sufficient for defining patient groups with or without pancreatic reaction or acute pancreatitis, the incidence considerably increased, but would not reflect a real pathological condition. Referral bias may also be taken into account as for example in our study, only patients with severe clinical presentation of infectious diarrhoea were referred and hospitalized in the Gastroenterology department. Nevertheless, the course of the pancreatic involvement in those patients exhibiting radiological features of acute pancreatitis was usually mild or moderate with few features at sonography or computed tomography and rapid clinical improvement. This low frequency occurrence of acute pancreatitis is in accordance with recent findings by Tositti et al. [27] reporting one patient with acute pancreatitis among 507 (0.2%) adult patients with acute gastroenteritis and Pezzilli et al. finding no patient (among 30 patients) who developed acute pancreatitis during the course of S. enteritis or S. typhimurium infectious diarrhea [28]. Rarely, severe complications related to non-typhoidal Salmonella have been reported; if they occur they may be rather the consequence of the injury to other organs (i.e. acute tubular necrosis due to rhabdomyolysis) than the result of the pancreatic involvement itself [20].

The pathophysiology of Salmonella-associated pancreatic enzymes alteration or acute pancreatitis is currently unknown. However, some hypothesis could be suggested, depending on the virulence of the bacteria, on the infectious dose as well as on the genetic makeup and immunological status of the host [31, 32]. Firstly, in case of important bacterial load, a transient bacteremia could occur allowing the infectious organism to spread to the reticuloendothelial system. The bacteria could then re-enter the bloodstream and potentially invade other organs during a second bacteremia leading for example to direct attack of the pancreatic acinar cells [33]. Direct pancreatic localization of the bacteria can also occur through transmural migration via the biliary duct system or from the duodenum via the pancreatic duct, a mechanism supported by some experimental results [34]. Secondly, non-typhoidal Salmonella may induce the production of inflammatory and immune mediators like arachidonic acid metabolites, nitric oxide, reactive oxygen species and pro-inflammatory cytokines [32, 35] through interaction with dendritic cells and/or macrophages [36-38] or by producing proteins secreted by a specialized type III secretion apparatus [39, 40] leading in part to NF-κB activation and subsequent pro-inflammatory cytokine genes transcription. Usually, these mediators participate in defence against invading agents [35]. However, depending on Salmonella strain virulence and/or host susceptibility, this immune response may exceed its physiological objectives and become detrimental, for example by increasing intestinal permeability [41] or favoring extra-intestinal manifestations [42]. Such an inappropriate immune response is suggested by the statistically significant increase in blood neopterin, sRIL2 and, despite less frequent, systemic pro-inflammatory cytokines concentrations in the PR group. Increase in pro-inflammatory mediators, for example in TNF-α, may also be accountable for hypertriglyceridemia. In addition, several authors reported an impairment of mucosal antioxidant defences during S. typhimurium infection which may contribute to the pathogenesis of the disease and the occurrence of extra-intestinal complications [43]. Another factor may be the type of the Salmonella strain: in fact, several strains

**Fig. 1** – Serum amylase ($r = 0.45, P < 0.001$, linear regression) (A), serum creatinine ($r = 0.58, P < 0.0001$) (B) and blood neopterin ($r' = 0.9, P < 0.01$, Spearman rank test) (C) were positively correlated with plasma triglyceride concentration.

Corrélations entre triglycéridémie et amylasémie ($r = 0.45, P < 0.001$, régression linéaire) (A), créatinémie ($r = 0.58, P < 0.0001$) (B) et taux sanguin de néoptérine ($r' = 0.9, P < 0.01$, Spearman rank test) (C).

contributing factors. Here we report a frequency of 24% (85.7% of them related to Salmonella strains) of increased pancreatic enzyme activity in the course of acute infectious diarrhea. These differences between our study and the above mentioned reports may be explained by differences in clinical and biological definition of pancreatic reaction and/or acute pancreatitis from one
have been considered to be more aggressive, thereby exhibiting a broader spectrum of clinical and biological symptoms compared to other strains [44]. In our series the most involved bacterial strains were Salmonella enteritidis (N = 6) and typhimurium (N = 2), and there is an obvious link between several inflammatory cytokines or markers of cellular immune activation and these strains. These data reinforce the previously discussed pathogenic mechanisms. In addition, reactive pancreatitis like reactive arthritis after infection with these enteropathogenic bacteria in genetically predisposed individuals may be a third explanation. As suggested by previous studies these syndromes may be linked to abnormal immunological reactivity against microbial antigens [45-49], therefore depending on hosts’ ability to fight against enteroinvasive bacteria. Finally, the severity of the infectious diarrhea (especially in the case of enteropathogenic microorganisms) may lead to severe dehydration with microcirculatory compromise explaining the pancreatic reaction. This hypothesis is in accordance with the higher urea and creatinine concentrations observed in the PR patients group as well as the longer duration of diarrhea, and has previously been suggested by Tosetti et al. [27] who found that pancreatic hyperamylasemia during acute gastroenteritis was significantly linked to the increased incidence of fever, dehydration, and a higher number of stool evacuations per day.

What about previous reports suggesting that hyperamylasemia and hyperlipemia in the course of infectious diseases were false positives? In fact, diseases complicated by renal failure may exhibit elevated serum amylase and lipase concentrations linked to the reduced renal clearance of these enzymes [50-53]. In such cases measurement of isoenzymes of amylase to determine its origin or concomitant assessment of pancreatic enzymes less susceptible to be affected by renal function (e.g. elastase I) may be helpful [54]. However, in this case, amylasemia or lipasemia usually do not exceed 2- to 4-fold the normal values, and renal insufficiency must be severe, which is not the case in our PR group. Other authors have suggested that hyperamylasemia and lipasemia may be the result of enhanced intestinal permeability as a consequence of mucosal inflammation, favoring the passage of these enzymes through the epithelium to the circulation [21, 27]. In fact, and supported by our results, there may be a close relation between inflammatory syndrome (immune cells activation, production of acute phase reactants and pro-inflammatory cytokines), alteration in lipid metabolism in relation to infection and/or renal insufficiency, and finally, pancreatic susceptibility to increased levels of circulating triglycerides. In the course of infectious diseases, hypertriglyceridemia has been occasionally observed as a result of increased synthesis and secretion of triglyceride-rich lipoproteins by the liver and the inhibition of lipoprotein lipase induced by bacterial endotoxins and several inflammatory mediators (i.e. TNF-α and IL-6) produced in response to infection [55-57]. Moreover, triglyceriderich lipoproteins are considered by several authors to participate in innate immunity by binding and/or neutralizing bacterial lipopolysaccharides [58, 59], but also by increasing the production of some cytokines or inflammatory mediators potentially involved in the development of acute pancreatitis [60]. As suggested by our data, hypertriglyceridemia may participate, at least in part, to the onset of pancreatitis as observed in other conditions associated with hypertriglyceridemia and complicated by pancreatic disturbances [61-63]. Furthermore, as stated previously, renal impairment may contribute to enhance plasma triglyceride concentration transiently by inhibiting the lipoprotein lipase activation [64, 65]. However, the role of hypertriglyceridemia in the onset of alteration in pancreatic enzymes or in the occurrence of acute pancreatitis in our patients group may probably be of less importance than in patients presenting hypertriglyceridemic pancreatitis. In these conditions, marked hypertriglyceridemia is necessary to induce pancreatitis [63], leading to toxic free fatty acid concentrations in pancreatic venules, with ischemic thrombosis [66]. The common elevation of hypofibrinolytic plasminogen activator inhibitor activity in severe hypertriglyceridemia would also contribute to increase the severity of triglyceride-induced pancreatitis, since thrombi, once formed, could not easily be hydrolyzed [66].

Finally, the occurrence of pancreatic reaction or pancreatitis in a patient presenting with diarrhea may lead to consider the possible diagnosis of inflammatory bowel disease (IBD). It is well known that some patients with IBD may develop acute [67] or chronic pancreatitis [68, 69]. In a recent population-based case-control study, Munk et al. [69] reported a 4-fold increase in risk for acute pancreatitis in patients with Crohn’s disease (CD) and a 1.5-fold increased risk for ulcerative colitis (UC). However, one should note that elevated pancreatic enzymes activity has also been described in IBD patients without any clinical or radiological signs of pancreatitis; in this study most of the patients with abnormal pancreatic enzymes had more extensive colonic disease and high histological activity [70]. Besides pancreatitis occurring as a side effect of drugs used in IBD (azathioprine or 6-mercaptopurine, aminosaliclyates, corticosteroids and metronidazole) or complicating local structural abnormalities (cholelithiasis, duodenal CD or primary sclerosing cholangitis), some cases are considered as “idiopathic”, and may then be regarded as true extraintestinal IBD manifestations [67]. In these cases acute pancreatitis does not necessarily occur during IBD onset or relapse [71] (differing from pancreatic reaction associated with infectious diarrhea which was present in all patients at the same time as acute diarrhea) and stool cultures were usually negative. The pathophysiology of IBD-associated acute pancreatitis remains incompletely understood. Direct granulomatous involvement of the pancreas seems to be a very rare condition [72, 73] and is not necessarily responsible for clinical or biological pancreatic abnormalities in these patients [73]. One experimental study suggests that pancreatic changes may be, at least in part, mediated by pancreatic inflammation [74]. In our series, to our knowledge, no patient developed IBD (in most of them, the follow-up exceeded one year; data not shown).

In summary, our observations indicate that patients with unusual upper abdominal pain in non-typhoidal Salmonella enterocolitis (or more rarely other forms of dysenteritic-like diarrhea) may be considered for concomitant alteration in pancreatic enzymes or true acute pancreatitis, and therefore appropriately investigated. The outcome of this rare complication is usually good. At present, the pathophysiological discussion remains open but our data and the literature suggest that pancreatic reaction may be the result of the virulence of particular bacterial strains which lead to inflammatory and immune changes responsible for a spectrum of metabolic alterations (hypertriglyceridemia, renal impairment, direct effect of inflammatory mediators on exocrine pancreatic cells, etc.). Nevertheless, the occurrence of true acute pancreatitis is a rare event (5.1% in our series) and, therefore, systematic screening in all patients presenting with infectious diarrhea would not be justified or recommended.

REFERENCES


