

ORIGINAL ARTICLE

Are Pancreatic Autoantibodies Associated with Azathioprine-Induced Pancreatitis in Crohn's Disease?

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ABSTRACT

Context Azathioprine is frequently used in the treatment of Crohn's disease. A severe side effect is acute pancreatitis, which is specific for Crohn's disease. Autoantibodies against exocrine pancreas occur in about 30% of Crohn's disease cases but not in other inflammatory diseases. Pancreatic autoantibody positive Crohn's disease patients might have a low grade inflammation of the pancreas which may be aggravated by the introduction of azathioprine, resulting in clinically overt acute pancreatitis.

Objective We hypothesized that the presence of pancreatic autoantibodies in Crohn's disease patients is associated with the development of azathioprine-induced pancreatitis.

Participants Eight patients with Crohn's disease and azathioprine-induced pancreatitis and 26 patients with Crohn's disease not using azathioprine.

Main outcome measure Pancreatic autoantibodies were determined by a standardized immunofluorescence method.

Results Two out of 8 patients with azathioprine-induced pancreatitis were positive for pancreatic autoantibodies (25.0%), detectable in serum dilutions of 1:40 and 1:160, respectively. In the control group of Crohn's disease patients, two (7.7%) were positive in serum dilutions of 1:2. All positive

samples had an extracellular fluorescence pattern. The difference in the prevalence of pancreatic autoantibodies was not statistically significant ($P=0.229$).

Conclusions We could not confirm our hypothesis that most or all patients with azathioprine-induced pancreatitis were pancreatic autoantibody positive. The prevalence of pancreatic autoantibodies in the Crohn's disease patients in our group was lower than in previous reports. This study does not support an association between pancreatic autoantibodies and azathioprine-induced pancreatitis in Crohn's disease. However, this association should not be definitively excluded and larger, preferably prospective, studies are needed.

INTRODUCTION

Crohn's disease is a chronic inflammatory disease involving the entire gastrointestinal tract. The etiology is thought to consist of an inappropriate immune response to commensal bacteria in the gut and a failure to downregulate this inflammatory response. Genetic and environmental factors play an important role in the pathogenesis. It is a chronic disorder characterized by periods of quiescence and periods of active disease [1]. Azathioprine is a purine analogue which is frequently used as maintenance therapy for

Crohn's disease. However, the occurrence of side effects is a major drawback in the use of azathioprine. In up to 23% of patients with IBD, azathioprine is withdrawn due to these side effects. It is increasingly more apparent that side effects occur more often in IBD than in other autoimmune diseases [2, 3]. One of the most severe side effects is the occurrence of acute pancreatitis which is seen in approximately 5% of patients with Crohn's disease and very rarely in patients treated with azathioprine for other diseases [2].

Polymorphisms in the gene encoding for thiopurinomethyltransferase (TPMT), which metabolizes azathioprine to 6-mercaptopurine and 6-methyl-mercaptopurine, have been associated with the development of dose-dependent side effects such as myelosuppression but not with azathioprine-induced pancreatitis [4]. A possible mechanism for the occurrence of side effects in azathioprine therapy might be explained by inosine triphosphate pyrophosphatase (ITP-ase) deficiency. In one study, flu-like symptoms, rash and acute pancreatitis were associated with a missense mutation in the gene encoding for inosine triphosphate pyrophosphatase [5]. However subsequent studies failed to confirm this association [6, 7]. Consequently, azathioprine-induced pancreatitis is a very severe side effect but the pathogenetic mechanism is unknown.

Circulating pancreatic autoantibodies (PABs) are found in approximately 30% of patients with Crohn's disease (Table 1) [8, 9, 10, 11, 12, 13]. PABs are not found in healthy

controls, in patients with other gastrointestinal diseases or in various autoimmune disorders (including autoimmune hepatitis, systemic lupus erythematosus and rheumatoid arthritis). Since PABs and azathioprine-induced pancreatitis are both specific for Crohn's disease, an association or a pathogenic role of PABs in azathioprine-induced pancreatitis could be expected. One pathogenic mechanism could be that azathioprine aggravates the inflammation in an already inflamed pancreas in Crohn's disease in PAB-positive patients. We evaluated the prevalence of PABs in patients with azathioprine-induced pancreatitis in Crohn's disease and in controls with Crohn's disease, hypothesizing that most or all patients with azathioprine-induced pancreatitis have circulating PABs.

MATERIALS AND METHODS

Patients

Patients were available from the IBD-cohort of the University Medical Center of Groningen [14]. Patients fulfilling the diagnostic criteria for Crohn's disease were eligible for the study. These criteria are based on accepted clinical, endoscopic, radiological and histological findings [1]. The diagnosis of azathioprine-induced acute pancreatitis was made by the attending physician according to the following criteria: upper abdominal or radiating pain with or without nausea and vomiting with at least a three-fold rise in serum amylase in combination with the disappearance of these symptoms after the withdrawal of azathioprine. All patients had an upper abdominal ultrasonography to exclude biliary pancreatitis. Patients with suspected alcohol-induced pancreatitis were excluded. Ten patients with azathioprine-induced pancreatitis were selected.

Based on the assumption that azathioprine-induced pancreatitis was associated with the presence of PABs, one could hypothesize that a control group of Crohn's disease patients using azathioprine but without pancreatitis would have a lower than expected prevalence of PABs. Therefore, the controls consisted of 31 randomly selected patients with Crohn's disease but not using azathioprine.

Table 1. Presence of pancreatic autoantibodies in Crohn's disease patients and healthy controls.

Author	Crohn's disease	Healthy controls
Stocker [8]	23/59 (39.0%)	3/100 (3.0%)
Seibold [9]	68/222 (30.6%)	0/65 (0%)
Folwaczny [10]	29/76 (38.2%)	2/54 (3.7%)
Joossens [11]	54/169 (32.0%)	0/100 (0%)
Klebl [12]	60/208 (28.8%)	0/50 (0%)
Koutroubakis [13]	32/77 (41.6%)	0/104 (0%)
Total	266/811 (32.8%)	5/473(1.1%)

Serum Samples

Serum samples were drawn from patients enrolled in the study as outpatients at the Department of Gastroenterology and Hepatology of the University Medical Centre of Groningen, the Netherlands and were stored at -80°C until analysis. Serum samples were obtained from 10 patients with azathioprine-induced pancreatitis (case group) and 31 patients with Crohn's disease not using azathioprine (control group). Two out of the 10 case samples (20.0%) as well as 5 out of the 31 control samples (16.1%) could not be evaluated due to the poor quality of the sera. Therefore, the sample of patients

evaluated in the study included 8 cases with azathioprine-induced pancreatitis and 26 controls with Crohn's disease not using azathioprine. Serum samples were obtained before the development of azathioprine-induced pancreatitis in two patients and subsequently in the other six patients with azathioprine-induced pancreatitis. The characteristics of the patients included with a successful PAB determination are shown in Table 2.

Pancreatic Autoantibodies

Autoantibodies to the exocrine pancreas were determined on frozen sections of human

Table 2. Clinical characteristics of the two groups of patients with Crohn's disease: patients with azathioprine-induced pancreatitis (cases) and patients not using azathioprine (controls).

	Azathioprine induced pancreatitis (Cases: n=8)	Patients not using azathioprine (Controls: n=26)	P value
Age (years) ^a	39 (21-57)	48 (32-64)	0.119 ^d
Gender:			0.422 ^e
- Males	2 (25.0%)	12 (46.2%)	
- Females	6 (75.0%)	14 (53.8%)	
Localization:			0.614 ^f
- Ileum	2 (25.0%)	11 (42.3%)	
- Colon	3 (37.5%)	6 (23.1%)	
- Ileocolon	3 (37.5%)	9 (34.6%)	
History of acute pancreatitis	0	0	1.000 ^e
Dosage of azathioprine (mg)^a	125 (70.6-179.4)	-	-
Time to onset (days)^a	24.5 (2.6-46.4)	-	-
Myelosuppression	0	-	-
Hospitalization	8 (100%)	-	--
Re-challenge with azathioprine	2 (25.0%)	-	-
Recurrence of symptoms	2 (25.0%)	-	-
Symptoms:			
- Upper abdominal pain	5 (62.5%)	-	-
- Nausea	5 (62.5%)	-	-
- Vomiting	3 (37.5%)	-	-
- Radiating pain	2 (25.0%)	-	-
- Fever	3 (37.5%)	-	-
Laboratory data (serum levels):			
- C-reactive protein (mg/L) ^{ab}	104 (4-126)	-	-
- Amylase (U/L) ^{ac}	829 (400-2,838)	-	-

^a Mean (range)

^b Reference range: 0-5 mg/L

^c Reference range: 0-100 U/L

^d Mann-Whitney U-test

^e Fisher's exact test

^f Chi-squared test

blood group 0 Rh- pancreata using an indirect immune fluorescence technique. The slides were incubated for 30 minutes with both an undiluted and a 1:10 diluted sample of the patient's serum. Binding of pancreatic autoantibodies was detected using fluorescein isothiocyanate (FITC)-labelled polyclonal rabbit anti-human IgG antibodies (F0202, Dakocytomation, Glostrup, Denmark). The slides were read under a fluorescence microscope (Leica DMRBE, Bensheim, Germany) by two independent observers. Serum samples showing either staining of the lumen of the acini (extracellular) or the cytoplasm of the exocrine cells (intracellular) were considered to be positive. There was 100% agreement for negative and positive samples between the two independent reviewers. Positive sera were diluted for semiquantitative interpretation of the results.[11, 12, 13, 15, 16].

ETHICS

Written informed consent was obtained from all participating subjects. The study protocol conforms to the ethical guidelines of the "World Medical Association Declaration of Helsinki", as reflected in a priori approval by the Medical Ethical Committee of the University Medical Center Groningen, the Netherlands.

STATISTICS

The sample size calculation, as well as the post-hoc evaluation of the power, were performed by using the PS Power and Sample Size Calculations Program Version 2.1.30 provided by the Department of Biostatistics of Vanderbilt University (<http://www.mc.vanderbilt.edu/prevmed/ps/index.htm>) [17]. The sample size calculation was made taking into account the assumption of a 0.85 prevalence of PABs in the Crohn's disease patients with azathioprine-induced pancreatitis and a 0.328 value in the control Crohn's disease group (Table 1). With a power of 0.80, a two-tailed significant level of 0.05 and a ratio of control to case patients equal to 3, a sample size of 10 patients with azathioprine-induced pancreatitis (together with 30 controls) was required.

The Fisher's exact, the chi-squared, and the Mann-Whitney U tests were applied by using the SPSS 14.0.2 statistical package (SPSS Inc., Chicago, IL, USA). Mean, range and frequencies were used as descriptive statistics.

RESULTS

Only two out of the eight patients with azathioprine-induced pancreatitis were positive for circulating PABs (25.0%); the titers were high being 1:40 and 1:160, respectively. These two samples were taken before the development of azathioprine-induced pancreatitis. None of the 6 samples which were obtained after the development of azathioprine-induced pancreatitis were PAB-positive. From the 26 serum samples of the controls, only two (7.7%) were positive for circulating PABs, both in low titers of 1:2. All positive samples had an extracellular fluorescence pattern. The prevalence of PABs in the two groups was not statistically significant having a P value of 0.229.

DISCUSSION

Since circulating PABs and azathioprine-induced pancreatitis are both specific for Crohn's disease, we hypothesized that the occurrence of PABs might be a risk factor for azathioprine-induced pancreatitis in Crohn's disease patients. We evaluated the occurrence of PABs in selected patients with azathioprine-induced pancreatitis and compared this with a control group of patients with Crohn's disease not using azathioprine.

Samples from 8 patients with azathioprine-induced pancreatitis were obtained and 25% of the patients with azathioprine-induced pancreatitis had circulating PABs *versus* 8% of the controls with Crohn's disease. Although there was a difference, this was not statistically significant. Moreover, there were no significant differences in clinical or demographic variables between PAB-positive and PAB-negative patients (data not shown). PAB titers in the controls were very low whereas PABs were detectable in high concentrations in the patients with pancreatitis. This may indicate that the presence of PABs at high titers increases the

risk of developing pancreatitis but is not a determining factor .

One study suggests that patients with Crohn's disease and pancreatic exocrine insufficiency were significantly more likely to be PAB positive than patients with Crohn's disease without pancreatic insufficiency [18]. The reason why Crohn's disease patients have pancreatic insufficiency is unknown, but it might be due to a low-grade inflammation of the pancreas, possibly as an extraintestinal manifestation of Crohn's disease. Furthermore, the use of azathioprine worsens the inflammation of the pancreas which has been shown in an animal model of acute pancreatitis in rats [19]. By extrapolating these findings, one could suggest that adding azathioprine to an already inflamed pancreas in PAB positive Crohn's disease patients aggravates the inflammation and leads to a clinical overt picture of acute pancreatitis.

Interestingly, we could not confirm the previously described prevalence of PABs in approximately 30% of the patients with Crohn's disease (Table 2) [8, 9, 10, 11, 12, 13]. In our population a total of 11.8% (4 out of 34) of the patients were positive for PABs. Although our sample size was small, the difference with each individual previous study is noticeable. Since PABs have not been shown to be associated with either disease severity or the use of medication and are stable in time, this difference is probably not caused by a selection bias. Furthermore, the antibodies were determined by a laboratory with extensive experience in immunofluorescent autoantibody testing, especially in the determination of islet cell antibodies for the purpose of type-1 diabetes diagnostics [15, 16]. The technique applied did not differ from what has been described in previous studies in Crohn's disease [11, 12, 13], thus indicating that the prevalence of PABs varies between Crohn's disease patient groups.

In recent years, many circulating antibodies have been identified in patients with inflammatory bowel disease. These antibodies have mainly been evaluated for the purpose of classification and the prediction of disease behavior [20]. The two most specific

serological tests for ulcerative colitis and Crohn's disease are perinuclear antineutrophil cytoplasmic antibodies (pANCA) and antibodies against *Saccharomyces cerevisiae* (ASCA), respectively [21]. In addition to PABs, pANCA and ASCA, various antibodies have been described including antibodies against outer-membrane porine C (OmpC) from *Escherichia coli* and, recently, serum reactivity to CBir1 flagellin has been identified in a subset of patients with complicated Crohn's disease [22, 23]. Whether these antibodies are of pathogenic relevance and directly cause tissue inflammation or are merely an epiphenomenon is a matter of debate.

A major limitation of this study was the poor quality of the sera. In fact, about one-fifth of the samples could not be used for PAB determination (2 patients with azathioprine-induced pancreatitis and 5 controls). We re-evaluated the statistical power by using the observed frequencies and the numbers of patients in our groups and this post-hoc analysis yielded a very low power of 0.155. Therefore, this study was underpowered for finding an association of PABs with azathioprine-induced pancreatitis; however neither can we disprove our hypothesis. Larger, preferably prospective, studies must be performed to clarify this. Moreover, due to the low prevalence of azathioprine-induced pancreatitis, this should be done in multiple centers.

CONCLUSIONS

We studied the occurrence of PABs in patients with Crohn's disease and azathioprine-induced pancreatitis and in controls with Crohn's disease not using azathioprine. Although the numbers were small, this study does not support an association between PABs and azathioprine-induced pancreatitis in Crohn's disease. However, due to the lack of power detected in the post-hoc analysis and the high titers of PABs found in patients with azathioprine-induced pancreatitis when compared to controls, neither can we rule out this association. Additional larger studies are

needed to clarify the pathogenetic mechanisms of azathioprine-induced pancreatitis in order to eventually enable clinicians to identify patients at risk.

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Keywords Antibodies; Azathioprine; Crohn Disease; Inflammatory Bowel Diseases; Pancreas; Pancreatitis; Serology

Abbreviations IBD: inflammatory bowel disease; PAB: pancreatic autoantibodies

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