Autoimmune Pancreatitis
Novel Insights on Diagnosis, Treatment and Outcome

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COLOFON

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CHAPTER 1A

AUTOIMMUNE PANCREATITIS
A REVIEW OF THE LITERATURE

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Submitted

INTRODUCTION

Autoimmune pancreatitis was first described in 1961[1]. It was not before 1995 that Yoshida[2] introduced the term autoimmune pancreatitis (AIP) for this rare form of pancreatitis. In 2001, a milestone was reached, when Hamano[3] discovered that serum IgG4 levels are elevated in AIP patients, providing a diagnostic marker to distinguish AIP from other pancreatobiliary disorders. Since then, this benign fibro-inflammatory disorder has been increasingly recognized[4-6].

Clinically, AIP mimics pancreatic cancer, with painless obstructive jaundice and weight loss as most common symptoms. Unlike other types of pancreatitis, AIP responds dramatically to steroid therapy[7,8]. Therefore, a correct and timely diagnosis is of utmost importance, to provide proper treatment and avoid complications and unnecessary interventions. Despite a strikingly favorable treatment response, initially, relapses are common, and maintenance therapy may be necessary to control the disease[9].

AIP has two subtypes[10,11]. Type 1 represents the pancreatic manifestation of an IgG4-related systemic disease (IgG4-RD), which can involve several other organs, such as the biliary tree, kidneys, and salivary glands[12]. It is the most common form, associated with elevated levels of serum IgG4, and relapses are frequently observed. Type 2 is pancreas-specific, not associated with elevated serum IgG4, and rarely causes relapse[13].

EPIDEMIOLOGY

Data on the incidence and prevalence of AIP are scarce and most studies originate from Japan[14,15]. There, a nationwide survey showed an overall prevalence of 2.2 per 100.000, with an annual incidence rate of 0.9/100.000. Worldwide, the distribution between type 1 and 2 AIP varies. In general, type 1 is more common, but this predominance is much more evident in Asia than in the USA or Europe[16-18]. Type 1 and 2 AIP have different demographics. Type 1 concerns mostly male patients, with a peak age of onset between 60 and 70 years[15]. Type 2 develops at a younger age (40, on average), and affects both sexes equally[16].

PATHOGENESIS

The pathogenesis of AIP is largely unknown. Although the presenting symptoms and response to therapy in type 1 and 2 AIP are similar, the two diseases have unique pancreatic histopathologic patterns, and, in contrast to type 2, type 1 AIP is part of a systemic disease. Therefore, despite their nomenclature, a distinct pathogenesis is suspected.

An immune mediated disorder was soon considered for both types, given the infiltration of plasma cells in the pancreas and the excellent response to steroid therapy. A likely mechanism in such disorders is that the disease develops in genetic susceptible persons after exposure to certain triggers. Different genetic associations have been reported for AIP, which suggest a genetic susceptibility for AIP[19-23]. Also, a relation with *H.pylori* infection was postulated, which assumes an autoimmune response via molecular mimicry in genetically predisposed persons[25,26]. However, these studies need confirmation. Because most causative studies focussed on the

more prevalent type 1 AIP, even less is known about the aetiology of type 2 AIP, which may be completely different.

A variety of autoantibodies have been demonstrated in sera of AIP patients, although, up to now, none appear to be disease specific[24]. For type 1 AIP and IgG4-RD, the role of IgG4 antibodies is still poorly understood. Whether elevated serum IgG4 is pathogenic or an innocent bystander of the inflammatory response remains unclear. Traditionally, IgG4 represents the least abundant subclass of IgG, typically less than 5% in healthy individuals. Although the normal value varies, it remains stable in healthy individuals[27]. IgG4 is regarded as a regulatory antibody, rather than fulfilling an inflammatory role. There are several other clinical conditions associated with elevated serum IgG4. In some, a protective effect is suggested, like maintenance of tolerance to cow's milk in atopic individuals, and in patients who underwent bee-venom immunotherapy after severe anaphylactic reactions[28,29]. There is one clinical condition described, pemphigoid diseases, where a clear pathologic role of IgG4 is described, as an IgG4 antibody to the epithelial antigen desmoglein causes blister formation[27].

HISTOLOGY

When two distinct histological patterns were recognized, it became clear that AIP concerns different subtypes[13,27]. Type 1 is characterized by a lymphoplasmacytic sclerosing pancreatitis

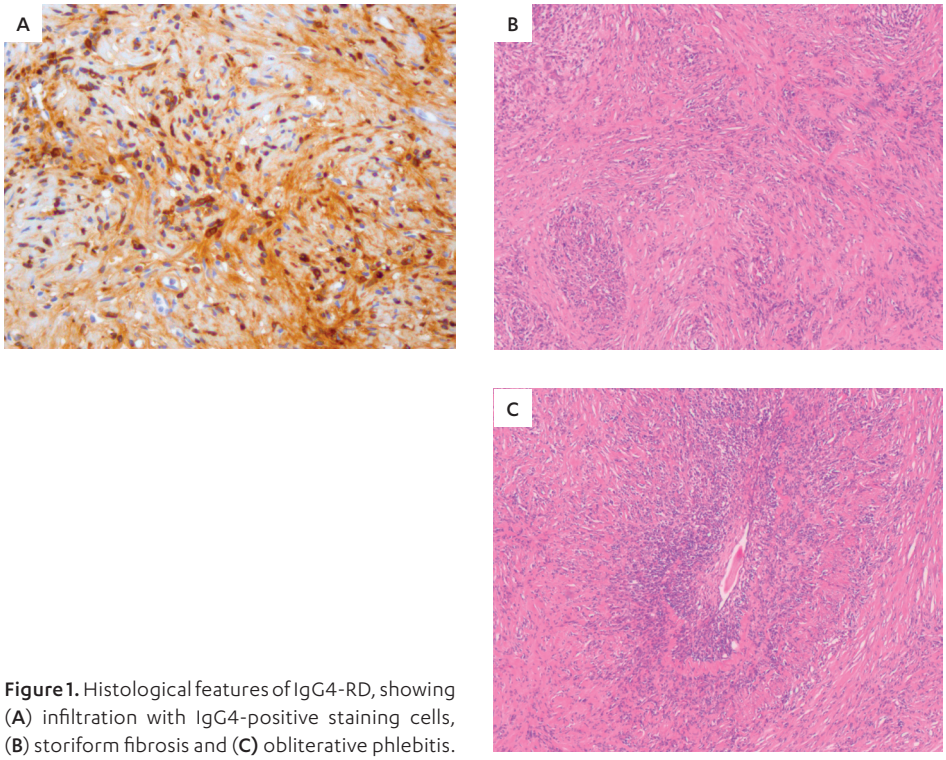

Figure 1. Histological features of IgG4-RD, showing (**A**) infiltration with IgG4-positive staining cells, (**B**) storiform fibrosis and (**C**) obliterative phlebitis.

(LPSP), which comprises of a combination of periductal lymphoplasmacytic infiltration, rich in IgG4, storiform fibrosis, and obliterative phlebitis (Figure 1). In type 2 AIP, an idiopathic duct-centric pancreatitis (IDCP) is recognized, with granulocyte epithelial lesions (GEL's). Obliterative phlebitis is less common in this type and IgG4-positive cells are scarce or absent.

DIAGNOSTIC CRITERIA

Establishing the diagnosis of AIP can be difficult, as a single diagnostic test is lacking. Instead, several diagnostic scoring systems are used to confirm the diagnosis. In 2006, the HISORt criteria were proposed by Chari et al., which are based on a combination of Histology, Imaging, Serology, Other organ involvement, and Response to therapy[6]. These criteria categorize patients in three groups, according to the diagnosis being based on; histology (group A); radiology, in combination with IgG4 positivity or other organ involvement (Group B); or unexplained pancreatic disease, IgG4 positivity, and a positive response to steroid therapy (Group C). Either typical histology or IgG4 positivity is mandatory to fulfil these criteria.

Next, the Asian criteria were introduced in 2008[28]. Compared to the HISORt criteria, they are more straightforward, requiring either a histological confirmation or typical radiological findings. Unfortunately, either way, invasive procedures are generally needed to provide certainty. First, to establish a histological diagnosis by fine needle biopsy (FNB) can be difficult, due to patchy distribution of the disease throughout the pancreas. Second, to establish a radiological diagnosis, an invasive endoscopic retrograde cholangio-pancreatography (ERCP) is often required to detect the subtle pancreatic duct changes.

Recently, an international panel of experts developed the International Consensus Diagnostic Criteria (ICDC)[29]. These criteria combine aspects of the HISORt and Asian criteria and categorize features as level 1 and 2 findings, to specify the level of evidence for a diagnosis of AIP. Furthermore, these criteria are the first to distinguish type 1 and type 2 AIP.

For clinical practice, the HISORt criteria are the most feasible. However, it is not possible to diagnose a subgroup of IgG4-negative AIP patients, in which histology is lacking. The ICDC overcome this problem and are the only diagnostic criteria that distinguish the two AIP subtypes. However, the ICDC are complex and therefore less suitable for daily practice. Also, as the definition of type 2 AIP is based solely on histology, which is not always available, they can lead to under-recognition of type 2 disease.

CLINICAL FEATURES

The clinical presentation of AIP is the same for both subtypes, with obstructive jaundice and weight loss as most common symptoms. Steatorrhea and diabetes mellitus are also frequently seen. Consequently, these patients are often wrongly suspected of having pancreatic cancer, resulting in invasive procedures or even major surgery[30]. For example, in 2% of patients who underwent a pancreatoduodenectomy for presumed pancreatic cancer, histology revealed AIP[31].

In type 1 AIP, other organs are involved in 45-52% of patients, including the biliary tract, salivary glands, and kidneys[9,12,17]. These manifestations show the same, characteristic infiltration

of IgG4-positive cells, leading to the term IgG4 related disease (IgG4-RD). Over the years, many new organ manifestations of IgG4-RD have been recognized. Although the pancreas and bile ducts are most commonly involved, the disease has been described in nearly every organ system. Other organ involvement can precede, present simultaneously, or follow the presence of pancreatitis. It may help establish the diagnosis of AIP, when these manifestations are more accessible for tissue collection. In type 2 AIP, other organs are rarely affected, but this type is associated with inflammatory bowel disease (IBD). The reported prevalence of IBD in these patients varies from 16 to 31%[9,18,32].

DIAGNOSIS

Serology

Up to now, elevated serum IgG4 (>140 mg/dL) is the best diagnostic marker for AIP, with a sensitivity of 76% and specificity of 93%[33]. However, generally, type 2 AIP patients do not present with an elevated serum IgG4, while 5-10% of patients with pancreatic cancer, acute or chronic pancreatitis do[9,34]. Therefore, serum IgG4 alone cannot be used to diagnose AIP.

Several other autoantibodies have been reported in AIP patients, and are even incorporated in the Asian diagnostic criteria. However, the clinical applicability of these antibodies in AIP has not been confirmed. A validation study in a Western population did not prove any of these autoantibodies useful in diagnosing AIP[35]. Recently, an Italian group identified a novel serologic antibody for AIP. This antibody, against *H.pylori* associated plasminogen-binding protein, yields a sensitivity of 94% and specificity of 95%, when comparing AIP with pancreatic carcinoma[26]. These promising results call for urgent validation in large (multicentre) cohorts.

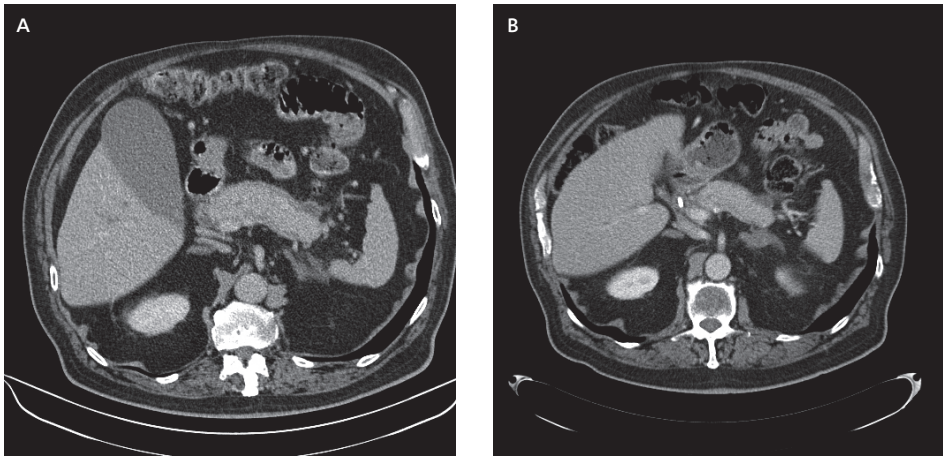

Figure 2. CT-scan showing (**A**) a diffusely enlarged pancreas with characteristic rim enhancement before steroid therapy and (**B**) response after steroid therapy.

Imaging

Imaging plays an important role in diagnosing AIP. To investigate the cause of obstructive jaundice, cross-sectional imaging is performed with computed tomography (CT) or magnetic resonance imaging (MRI). In the active phase of the disease, a diffusely enlarged pancreas is often found, sometimes with a characteristic rim enhancement[36] (Figure 2A). Some AIP patients present with a pancreatic mass, which obviously makes it more difficult to distinguish AIP from pancreatic cancer[37]. Furthermore, in patients who present late in the course of the disease, especially after multiple undiagnosed relapses, pancreatic atrophy can be found.

Histology

The gold standard to diagnose AIP is pancreatic histology. Histology can be obtained through biopsy or surgery. In resection specimens, a reliable diagnosis can be easily made. But given the excellent medical treatment response, surgery is to be avoided in patients with AIP. Fine needle aspiration (FNA) is a sensitive method to diagnose pancreatic cancer[38], but the diagnostic value for AIP is less good[39]. Due to the patchy distribution of characteristic histological features, negative results are difficult to interpret[40]. However, the technique of pancreatic tissue acquisition is evolving and experienced endoscopists have reported to achieve better results with endoscopic ultrasonography (EUS)-guided biopsy (FNB) [41,42]. Therefore, future studies should determine the use of FNB in autoimmune pancreatitis.

TREATMENT

Induction of remission

The first therapeutic step in AIP is to induce clinical remission with steroids. Up to now, there is no consensus on the precise definition of clinical response. Objective outcome measures such as resolution of jaundice, biochemical improvement of liver function tests, and resolution of pancreatic or biliary duct strictures, or other pancreatic abnormalities on cross-sectional imaging, should be carefully monitored. Often, the first response is striking, with jaundice resolving in days. However, normalisation of liver enzymes values can take longer and bile duct strictures and other morphologic abnormalities may take several months to subside (Figure 2). Furthermore, elevated levels of IgG4 often decrease, but fail to normalise in the majority of patients[8].

Both type 1 and 2 patients show a nearly 100% response to steroid therapy[8,16,18]. Therefore, response to steroids is even used as a diagnostic tool to differentiate AIP from pancreatic cancer, and is incorporated in most diagnostic criteria[6,28,43]. A 2-week steroid trial can be useful, without negative consequences for resectable pancreatic cancer, but predefined response criteria should be strictly applied[44].

The optimal steroid dosage for remission induction has not been established, as prospective studies are lacking. The most advocated treatment schedule consists of prednisone in a dosage of 30 to 40 mg per day for 2-4 weeks, after which steroids are tapered and stopped in 2-3 months[8,16]. Comparable outcomes were reported with 25 to 50 mg of prednisone, although these studies were not primarily aimed to evaluate dose-response relationships[8,45-47]. Reports on

corticosteroid induction doses lower than 15 mg/d are scarce. One case report even described successful treatment with a prednisolone dosage of 5 mg/d[48]. Future studies are needed to define the optimal treatment regimen.

Relapses and maintenance therapy

Despite a successful initial treatment response in the vast majority of patients, 27 to 47% of patients with AIP develop a recurrence[8,9,18]. Relapses typically occur in the pancreas and/or bile ducts, but can also arise in other involved organs. Recurrences are more common in type 1 AIP patients and in patients with a concomitant IgG4-associated sclerosing cholangitis[9,18]. Generally, relapses are treated with a second course of steroids, to which most patients again respond favourably.

To prevent relapses, low-dose steroids can be used for maintenance. However, long-term steroid treatment may result in significant side effects, especially in the elderly. As an alternative, immunomodulators, such as azathioprine, can be prescribed. Conflicting results of azathioprine have been reported, from treatment-limiting side effects to successful use without serious complications[18,49-52]. Therefore, future studies are needed to establish the role of azathioprine in AIP.

An argument against maintenance therapy in general is that, although relapses frequently occur, the majority of patients never develop a recurrence. Furthermore, most recurrences are easy to treat. Therefore, the drawbacks of maintenance therapy may out way it's benefits. Perhaps, maintenance therapy will eventually prove valuable only in a subgroup of patients with an already established (or increased risk for) relapse. Recently, rituximab has been suggested as an alternative for patients with difficult-to-treat, relapsing AIP[49]. An open label trial in the USA is presently undertaken[53].

LONG-TERM OUTCOME

Little is known about the long-term outcome of AIP patients. Although AIP does not seem to affect overall survival[9], several long-term consequences have been suggested. As patients are often wrongly suspected of pancreatic cancer, resulting in invasive (diagnostic) procedures or even major surgery, it is possible that AIP has a negative long-term influence on quality of life. Furthermore, a delayed diagnosis may lead to pancreatic function loss, because prolonged inflammation causes fibrosis and pancreatic acinar and islet cell loss[18,54]. Another possible, late complication is the development of cancer. Other forms of pancreatitis are known to increase the risk for pancreatic cancer and the same association was reported for AIP[55,56]. Recently, it was also suggested that AIP leads to an increased risk for cancer of any origin[57]. Therefore, long-term outcome studies are needed to establish whether AIP patients are truly at risk.

In conclusion, AIP is a rare type of chronic pancreatitis, clinically mimicking pancreatic cancer, with a dramatic response to medical therapy, *ic*. steroids. In the last decade, AIP has been increasingly recognized, but despite recent advancements, there are still considerable gaps in our knowledge regarding its etiology, treatment, and long-term outcome.

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CHAPTER 1B

AIMS AND OUTLINE OF THE THESIS

AIMS

In the past decades, knowledge regarding autoimmune pancreatitis (AIP) has been rapidly evolving. However, much remains to be discovered about this intriguing form of pancreatitis.

The general aim of this thesis is to study the diagnostic process, treatment, and outcome of AIP. For this purpose, information is subtracted from an AIP database registry, that was established by three tertiary referral centers (Rotterdam, Amsterdam, Utrecht), in cooperation with several other hospitals. We describe the characteristics of this multicenter national cohort and evaluate outcomes.

OUTLINE

Establishing the diagnosis of AIP can be difficult, as a single diagnostic test is lacking. Over the years, several diagnostic scoring systems have been proposed, based on a combination of histology, imaging, serology, other organ involvement and response to therapy. In **chapter 2** we evaluate the performance of the three major diagnostic AIP scoring systems in our AIP cohort of 114 patients.

Due to the clinical presentation of AIP, with obstructive jaundice, weight loss and diabetes mellitus, patients are often wrongly suspected of having pancreatic cancer. This frequently results in invasive procedures or even major surgery. The tumor marker carbohydrate antigen 19-9 (Ca 19-9) is associated with pancreatic carcinoma, as elevated serum IgG4 is with AIP. In **chapter 3** we evaluate the diagnostic use of Ca 19-9 and serum IgG4 levels, in differentiating between AIP and pancreatic carcinoma. Furthermore, the two outcomes are combined, to potentially improve diagnostic performance.

Up to now, elevated serum IgG4 (>140mg/dL) is the best diagnostic marker for AIP. However, particular caution is needed when interpreting mildly elevated levels of IgG4, since they are also encountered in other pancreatobiliary disorders. In **Chapter 4**, we compare serum IgG4 levels of patients with acute, chronic, and autoimmune pancreatitis, to assess the diagnostic value in differentiating between these three forms of pancreatitis.

In 2009, an Italian group identified a novel serologic marker for AIP, with an outstanding sensitivity and specificity. The promising results of this marker, anti-PBP antibodies, have never been confirmed. In **chapter 5** we aim to validate the usefulness of this novel marker in AIP and various other pancreatobiliary diseases.

IgG4-related disease is a systemic disease and its manifestations have been described in nearly every organ system. Although the pancreas and bile ducts are most commonly involved, precise history taking is important to detect manifestations in other organs. In **chapter 6** we describe the occurrence and histopathological characteristics of IgG4-related prostatitis in our cohort of AIP patients.

The first therapeutic step in AIP is to induce clinical remission with steroids. Often, the first response is striking, with jaundice resolving in days. The optimal steroid dosage for remission induction has not been established, as prospective studies are lacking. Currently, a dosage of 30-40 mg per day is commonly used, although a lower dose would potentially reduce side-

effects. In **chapter 7**, we compare the efficacy of a high versus a low prednisone dose to induce disease remission.

Little is known about the long-term outcome of AIP patients. A delayed diagnosis may lead to pancreatic function loss, and like chronic pancreatitis, pancreatic cancer may be a late complication. The influence of AIP on quality of life is unknown and whether AIP affects survival remains unclear. In **chapter 8** we investigate the long-term impact of AIP, in terms of treatment response, pancreatic function, quality of life, incidence of malignancies, and mortality.

Finally, in **chapter 9**, we summarize and discuss the main findings of this thesis. In addition, we present directions for further research.

CHAPTER 2

A COMPARATIVE STUDY OF DIAGNOSTIC SCORING SYSTEMS FOR AUTOIMMUNE PANCREATITIS

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ABSTRACT

Objective

Several diagnostic scoring systems for autoimmune pancreatitis (AIP) have been proposed including the Asian, HISORt (Histology, Imaging, Serology, Other organ involvement and Response to therapy) and International Consensus Diagnostic Criteria (ICDC), which have been compared by a few studies. We evaluated the diagnostic performance of these criteria in patients diagnosed with AIP between May 1992 and August 2011.

Methods

Scoring systems were applied retrospectively using data obtained in the initial evaluation period, before pancreatic resection was performed.

Results

114 cases with AIP were included. 82% met the diagnostic criteria for AIP according to either the Asian, HISORt or ICDC criteria. Only 33% met the Asian criteria, probably mainly related to a low rate of diagnostic pancreatography. In 18% all scoring systems failed to confirm the diagnosis, even though these patients were considered to have a firm diagnosis of AIP.

Conclusions

In this cohort of AIP patients, the three major diagnostic scoring systems for AIP proved to be complementary rather than overlapping. Our data indicate that one-fifth of our AIP patients do not meet any of these scoring systems. The ICDC, Asian and HISORt criteria should be considered as useful clinical tools but not as gold standard for the diagnosis.

INTRODUCTION

Autoimmune pancreatitis represents the pancreatic manifestation of IgG4-related sclerosing disease that may affect not only the pancreas but also other organs including the extrapancreatic biliary tract, salivary glands, retroperitoneal tissues, and kidneys[1]. Since AIP often presents in men in their 6th or 7th decade, with jaundice, weight loss and a pancreatic mass, the differential diagnosis is focused mainly on malignancy. Recently, the Honolulu consensus meeting agreed on the presence of two distinct histological and clinical types of AIP: lymphoplasmacytic sclerosing pancreatitis (LPSP) / type 1 and idiopathic duct-centric pancreatitis (IDCP) / type 2[2-4]. Whereas type 1 represents the classical phenotype as described above, type 2 patients present younger (4th decade), without male preponderance. Abdominal pain and acute pancreatitis are observed more often. IgG4 elevation is rarely observed. Inflammatory bowel disease is often associated, other organ manifestations are rare. Response to steroids is good, with less recurrence (40-50% in type 1, 0-25% in type 2) [1,3-6]. The histological hallmark of type 2 is destruction of ductal epithelium by GELs (granulocyte epithelial lesions). Fibrosis is less common and IgG4 positive cells are scant or absent. Since there is no serological marker and other organ involvement (OOI) is uncommon, the autoimmune nature of AIP type 2 is a matter of debate[2].

There is no single diagnostic test to diagnose AIP, but diagnosis can be confirmed using diagnostic scores combining radiological (diffuse or focal enlargement, occasionally with rim enhancement, diffuse or segmental narrowing of the main pancreatic duct), histological (LPSP and IgG4 staining in type 1 AIP, GELs in type 2 AIP), serological (IgG4, IgG and the presence of autoantibodies) and other criteria such as involvement of other organs (sclerosing cholangitis, interstitial nephritis, retroperitoneal fibrosis or Sjögren-like syndrome) and response to steroid therapy[7-9]. The Asian criteria (2008)[8] and HISORt criteria (acronym for Histology, Imaging, Serology, Other organ involvement and Response to therapy, Mayo Clinic, 2006[7], revised in 2009[10]) are used most frequently. The Asian criteria are relatively easy to apply but require diagnostic pancreatography. Diagnosis is made in combination with either serology, histology or response to therapy. The HISORt criteria, categorizing patients in three groups, enable diagnosis solely based on histology, which gives special significance to pancreatic core biopsy (group A). Other ways to make diagnosis are: radiology in combination with IgG4 positivity or other -histologically proven - OOI (group B), and unexplained pancreatic disease, IgG4 positivity and response to therapy (group C). In group B and C, IgG4 positivity is mandatory. Recently, the ICDC were developed, based on aforementioned systems in combination with proposed Italian[6] and German criteria[11].

For use in daily clinical practice, these systems harbor their own pros and cons. The Asian criteria are easy and straightforward to use but a pancreatogram is always required. This contrasts with common European and American gastroenterology practice in which in case of suspected pancreatic cancer, early surgery is preferred over endoscopic retrograde cholangio-pancreatography (ERCP) / biliary drainage[12]. Moreover, to avoid post-ERCP pancreatitis, gastroenterologists usually do not attempt to deliberately cannulate and fill the pancreatic duct[13]. A non-invasive modality such as magnetic resonance cholangio-pancreatography (MRCP) is still inferior to ERCP in detecting the subtle ductal changes in AIP[14]. HISORt criteria

are somewhat more complicated but quite elegant, enabling a diagnosis of AIP from different angles. The ICDC are very elaborate. They encompass all kinds of difficult diagnostic situations which might improve sensitivity, but consequently complicate its application. This renders ICDC unsuitable for use in routine daily practice. The use of histology based criteria, part of all systems, poses some limitations and challenges. Histology obtained with resection is considered gold standard, but actually represents failure of the system to timely diagnose AIP, that is before resection. Histology obtained with endoscopic ultrasonography (EUS) core biopsy, although very attractive in expert hands[15,16], is limited by sampling error, is not routinely available in daily gastroenterology practice and requires a pathologist familiar with the diagnosis and IgG4 staining.

For these reasons we aimed to investigate the diagnostic performance of all three scoring systems, using the data on initial clinical presentation, with emphasis on preoperative data.

MATERIALS AND METHODS

Patients diagnosed with AIP between May 1992 and August 2011 were enrolled in this multicentre retrospective study. They were included if the ICDC, Asian or HISORt criteria were fulfilled. In addition, patients were eligible when (1) post-surgery pancreatic histology allowed an unequivocal diagnosis of AIP or when otherwise unexplained pancreato-biliary disease or extrapancreatic manifestations were diagnosed in combination with either (2) response to steroids or (3) IgG4-positive serology. Clinical data, laboratory and imaging findings, histology, response to treatment, and recurrence were studied to characterize the patient population. The radiological data were reviewed by a radiologist, expert pancreatologist and research fellow independently. In case of disagreement, cases were reviewed by the entire panel. Histological evaluation was performed by two expert pathologists familiar with IgG4 staining and specialized in hepatic, pancreatic and biliary pathology. If not performed already, immunostaining was performed using a monoclonal mouse anti-human IgG4 (Zymed Laboratories, San Francisco, USA), with a working dilution of 1:100. IgG4 positivity was defined as the presence of >10 IgG4-positive plasma cells in at least one HPF at a magnification of x400. Response to treatment was defined as resolution or marked (>50%) improvement of radiological, clinical and biochemical abnormalities, recurrence as reappearance of disease manifestations. Scoring systems were applied using data obtained during the initial evaluation period of six months. The onset of the initial evaluation period was defined as the presentation with major symptoms such as obstructive jaundice or overt pancreatic disease, prompting particular diagnostic activity. The clinical course of AIP may be protracted and highly variable, which may cause substantial diagnostic delay. Pancreatic resection because of presumed malignancy, although sometimes unavoidable, represents failure of timely diagnosis. None of the patients that underwent resection were operated later than six months after onset of jaundice or overt pancreatic disease. Therefore, data were used which were obtained during an initial evaluation period of six months, or until resection was performed.

One sample student t test and Fisher's exact test were used to compare differences in means or frequencies. A two-sided p-value ≤ 0.05 was considered statistically significant.

Medical ethical concerns

The study was approved by the institutional review board of the Erasmus University Medical Center, Rotterdam, the Netherlands.

RESULTS

Patient characteristics and clinical presentation

A total of 114 patients were included (Table 1). The median age was 62 (IQR 51-69) years, 99/114 (87%) were men. Females were diagnosed at a younger age than men (57, IQR 39-67 years, $p < 0.001$). Obstructive jaundice and signs of pancreatic exocrine insufficiency (weight loss and steatorrhea) were the most frequent presenting symptoms, followed by abdominal pain (usually mild discomfort) and recent onset diabetes (six months prior to diagnosis).

Other organ involvement (OOI) was present in two-thirds (78/114, 68%) of the patients. In two-thirds (52/78, 67%) of these patients one organ was affected, in one-third (26/78, 33%), multiple systems were involved. Usually, OOI coincides with or follows the pancreatic manifestation (34/78, 44%; 30/78, 38% respectively). In 18% (14/78) however, OOI was heralding AIP. Sclerosing cholangitis (65/105, 62% of all recorded OOI), was by far the most frequent extrapancreatic manifestation, followed by salivary gland involvement (Sjögren like syndrome, 9/105, 9%), retroperitoneal fibrosis (10/105, 10%), interstitial nephritis (5/105, 5%), localized or generalized lymphadenopathy (6/105, 6%), prostatitis (5/105, 5%), pulmonary involvement (4/105, 4%) and uveitis (1/105, 1%).

Elevated IgG4 (>1.40 g/L) was present in 82% (85/104). Levels above 2.8 g/L, which is the recommended level to discriminate between AIP and pancreatic cancer[10,17], were present in 60% (62/104). The tumor marker Ca 19-9 was elevated (>34 U/ml) in 58% (42/72). Levels above 300 U/ml, considered specific for pancreatic cancer[10,18], were present in 18% (13/72). Autoantibodies (antinuclear antibody, rheumatoid factor, pANCA, anti lactoferrin or anticarbonic anhydrase II) were found in less than one-third of patients.

Radiological findings

Radiological findings at initial clinical presentation are shown in Table 2. On computed tomogram (CT), diffuse swelling of the pancreas was present in 56% (63/113), focal enlargement in 33% (37/113), of which 30% (34/113) in the head and 3% (3/113) in the tail. In 25% (28/113) there was no enlargement. Rim enhancement, an important criterion in the HISORt system, was present in 24% (26/107). In 24% (27/113) regional adenopathy was noted, which could be misinterpreted as lymphatic metastases. In 24 patients (21%), abdominal CT showed no pancreatic abnormalities. Pancreatography (ERCP or MRCP) was performed in half of the patients (58/114, 51%). Diffuse stricturing of the main pancreatic duct was present in 66% (38/58), a segmental stricture in 17% (10/58). Ten percent (6/58) showed a normal pancreatogram. Biliary strictures were very common, mostly strictures of the intrapancreatic part of the common bile duct (84/97, 87%). Proximal biliary strictures (proximal hepatocholedochal, hilar or intrahepatic) were present in 34% (33/97).

Table 1. Baseline characteristics

		Patients n = 114	Percentage %
Demographic findings			
	Male gender	99/114	87
	Age, median (IQR), y*	62 (51-69)	
	Male	62 (53-70)	
	Female	75 (39-67)	
Presenting symptoms			
	Obstructive jaundice	87/114	76
	Abdominal pain	61/114	54
	Weight loss	98/111	88
	DM recent onset	40/113	35
	Steatorrhea	77/103	75
Serological findings			
IgG	>18.0 g/L	38/103	37
IgG4	Median (IQR) g/L	5.01 (1.73-9.55)	
	Normal < 1.40 g/L	19/104	18
	>1.40 g/L	85/104	82
	>2.80 g/L	62/104	60
Ca 19.9	>34 U/ml	42/72	58
	>100 U/ml	27/72	38
	>300 U/ml	13/72	18
Rheumatoid factor +		11/39	28
Antinuclear antibody +		22/71	31
Other antibody [Δ]		8/64	13
Other organ involvement			
Presence	None, n (%)	36/114	32
	Single, n (%)	52/114	46
	Multiple, n (%)	26/114	23
Timing [¥]	Preceding, n (%)	14/78	18
	Same time, n (%)	34/78	44
	Later, n (%)	30/78	38
Prior treatment			
	Resection	18/114	16
	Exploratory surgery [ˈ]	16/114	14

Table 1. Baseline characteristics (*Continued*)

		Patients n = 114	Percentage %
	Biliodigestive anastomosis	2/114	2
	Liver-kidney transplantation	1/114	1
	Chemoradiation	1/114	1
Diagnostic delay			
	median (IQR), months[ˈ]	4.3 (2.0-18.8)	

*Age at time of initial symptoms; [Δ]pANCA, ALF or ACA-II; [¥]with respect to onset of jaundice or overt pancreatic disease; [ˈ]explorative laparotomy, diagnostic laparoscopy; [ˈ]time between date diagnosis and date symptoms

Table 2. CT and MRCP/ERCP findings of AIP

		Patients n =114	Percentage %
CT			
Swelling of the pancreas:			
	Diffuse	63/113	56
	Segmental (head)	34/113	30
	Segmental (body)	0/113	0
	Segmental (tail)	3/113	3
	No enlargement	28/113	25
Rim enhancement		26/107	24
Pancreatic atrophy		6/113	5
Pseudocyst		2/113	2
Calcifications		1/113	1
Regional adenopathy		27/113	24
ERCP/MRCP			
Pancreatic duct:			
	Normal	6/58	10
	Stricture diffuse	38/58	66
	Stricture segmental	10/58	17
Biliary ducts:			
	Normal	3/97	3
	Stricture intrapancreatic	84/97	87
	Proximal (extrapancr) strictures	33/97	34
	Combined strictures	20/97	21

CT: computed tomogram; ERCP: endoscopic retrograde cholangio-pancreatography; MRCP: magnetic resonance cholangio-pancreatography

Histopathological findings

Histology was available in 35/114 patients (31%), mainly from resection specimens (17/114, 15%) and exploratory surgery biopsy (11/114, 10%). A diagnosis of AIP was histologically confirmed in all of them. In only 7/114 patients (6%) EUS fine needle biopsy (FNB) had been performed with lymphoplasmacytic sclerosing pancreatitis (LPSP) present in four and IgG4 positivity in two. FNB contributed to diagnosis in only one patient. AIP type 2 (reflected by idiopathic duct-centric pancreatitis IDCP), was diagnosed in 3/114 patients (3% of the entire cohort), all of which underwent pancreatic resection.

Therapy and prognosis

Steroid therapy was started in 95/114 (83%), with an excellent response rate of 98% (92/94). Reasons to refrain from steroids (19/114, 17%) were: resection without postoperative recurrence (n=7), spontaneous remission or relatively mild symptoms (n=8), or patient refusal (n=2). One patient died of cardiovascular disease before therapy was initiated. Recurrence was noted in 37% (41/111); after remission without treatment in 17% (19/111), during treatment (failure to wean) in 11% (12/111) and after treatment in 9% (10/111). Death occurred in 14% (14/99). In 29% (4/14) of the deaths the cause was disease related (cholangitis / sepsis, hepatic or renal failure), 29% (4/14) cardiovascular and 36% (5/14) unknown. One case of malignancy was recorded (acute leukaemia).

Performance of diagnostic scoring systems and basis diagnosis

Twenty of 114 patients (18%) met the diagnostic criteria for all three systems, 40/114 (35%) met criteria for two systems and 33/114 (29%) for one system (Table 3). In 18% (21/114) all three scoring systems failed to confirm the diagnosis of AIP, even though these patients had an unchallenged clinical diagnosis of AIP based on postoperative histology, a combination of unexplained pancreatic disease, biliary disease/extrapancreatic manifestations and either response to steroids or IgG4-positive serology.

Table 3. Basis on which diagnosis of AIP was confirmed

	Percentage (%)	No. patients
Positive for AIP criteria	82	93/114
3x positive	18	20/114
2x positive	35	40/114
1x positive	29	33/114
Negative for AIP criteria	18	21/114
(1) Histology	33	7/21
(2) Unexplained pancreatic disease +biliary disease/OOI + response to steroids	38	8/21
(3) Unexplained pancreatic disease + biliary disease/ OOI + IgG4 positive	38	8/21

OOI: other organ involvement

Table 4. Performance of diagnostic scoring systems at initial presentation

	Overall (n=114)	Pancreatogram† (n=58)	IgG4 negative (n=19)	Normal CT‡ (n=24)
HISORt	52%	53%	0%	50%
Group A	1%	0%	0%	0%
Group B	6%	12%	0%	0%
Group C	45%	41%	0%	50%
Asian	33%	62%	42%	0%
ICDC	68%	67%	58%	0%
AIP type 1 definitive	60%	55%	26%	0%
AIP type 1 probable	2%	0%	0%	0%
AIP type 2 definitive	0%	0%	0%	0%
AIP type 2 probable	0%	0%	0%	0%
AIP not otherwise specified	6%	12%	32%	0%

Systems were scored with initial clinical findings; that is, EUS FNA was included, histology obtained by resection or laparotomy biopsy was excluded. †Pancreatogram present; ‡Normal appearance of pancreas on CT.

The highest percentage of patients met the ICDC (77/114, 68%), followed by HISORt criteria (59/114, 52%, p=0.022) and Asian (37/114, 33%, p= 0.005, Table 4). The relatively poor performance of the Asian criteria in the entire cohort, was mainly due to a low percentage of diagnostic pancreatography in this series (58/114, 51%). If a pancreatogram was available, Asian, ICDC and HISORt criteria performed equally well (36/58, 62% vs 39/58, 67%, p=0.698 and 31/58, 53%, p=0.452, respectively). If abdominal CT showed no pancreatic abnormalities (n=24, 21%), HISORt was the only scoring system that could establish a diagnosis of AIP (group C, which requires response to steroid therapy). If IgG4 levels were normal (19/104, 18%), AIP was never diagnosed according to the HISORt criteria, while the Asian criteria and ICDC established the diagnosis in 42% (8/19) and 58% (11/19), respectively (p=0.517).

DISCUSSION

In this well-characterized cohort of AIP patients, the three major diagnostic criteria systems proved complementary rather than overlapping. At initial clinical presentation, the majority of patients with AIP was correctly identified by any of the three systems, without the need for histology. Our data further suggest that a small subset of patients does not meet the criteria of any of these systems. Therefore, these scores are valuable and helpful, particularly for defining populations, but should not be regarded as absolute, gold diagnostic standard. Based on our data and the clinical applicability of the respective systems, we recommend the use of HISORt criteria, and the Asian criteria if a pancreatogram is available (optional) or if IgG4 levels are normal (mandatory). If diagnosis is still not confirmed, the ICDC can be used.

In our opinion, the intrinsic erratic nature of AIP, with its protracted and highly variable clinical course, forms the main drawback in the clinical applicability of any diagnostic criteria system in a given moment of the disease. Radiological, clinical and biochemical abnormalities may fluctuate in time. The 18% of patients in our cohort that did not meet any of these systems in the initial evaluation period, had a firm diagnosis of AIP. It was only a matter of timing, pancreatic resection or steroid trial, to finally make the diagnosis. The main differential diagnosis with malignancy however, prompts the clinician to minimize diagnostic delay. We believe it is highly unlikely that any diagnostic criteria system will totally cover this dilemma in a given short period of time with sufficient specificity.

In general, our series resembles other large cohorts of AIP patients[10,19-22]. Since there were only three cases of histologically confirmed IDCP (type 2 AIP), our cohort reflects mainly the clinical spectrum of LPSP (type 1 AIP). Differences in disease characteristics between various cohorts are probably due to differences in the use of diagnostic criteria systems and their ability to differentiate between type 1 and type 2 AIP[2,3]. The Asian criteria and HISORt in particular highlight the features of AIP type 1. Italian[6] and German criteria[11] appear to be targeted at both types. Whether the good performance of the HISORt criteria in our cohort reflects selection bias or true low incidence of type 2 AIP in the Netherlands is not clear.

We report a high percentage of elevated Ca 19-9 levels in 58% of cases compared to an average of 25% in other series[6,10,21]. Levels above 300 U/ml, considered specific for pancreatic cancer[10,18], were present in 18%. This is not explained by a difference in the presence of proximal biliary involvement, which was similar (34%). We report five cases of AIP associated prostatitis, which is quite uncommon. No case of inflammatory bowel disease was noted, corresponding with virtual absence of type 2 AIP. Response and recurrence rates (98% and 37% respectively) match those reported in the literature.

To our knowledge, this is the first study that uses a well-defined cohort of AIP patients to head-to-head test the dominant and currently available diagnostic criteria systems for AIP. The strengths of our study include the large number of patients and the use of data at initial clinical presentation, which provides insight in the respective performance of these systems in common gastroenterology practice. Furthermore we provide specific clues for certain difficult diagnostic situations such as a normal CT (which was present in 21% of our cohort) and normal IgG4 levels (which were reported in 18%). One limitation of the current study is the retrospective nature and the use of HISORt and Asian criteria (that particularly highlight type 1), which might have introduced selection bias. Another limitation is the limited availability of pancreatic core biopsies, as they are only rarely obtained in the Netherlands. In only one of seven patients that underwent EUS fine needle biopsy (FNB) in our cohort did this contribute to diagnosis. Although the technique is increasingly feasible due to the development of flexible large bore EUS needles, the required histological expertise is not commonplace. This impairs a proper evaluation of its contribution to the diagnostic process in patients suspected of having AIP. However, the crucial role of EUS FNA (or FNB) in the diagnostic process of a pancreatic mass remains undisputed. Response to steroid therapy as a diagnostic tool to confirm AIP should only be used if malignancy is properly excluded[10], that is by an attempt to confirm malignancy by tissue sampling. A final limitation in our study is the observer bias that might have been

introduced in the radiological revisions. Only the radiologist was blinded for the diagnosis. In daily practice, pancreatic enlargement and rim enhancement can be quite subtle. The quality of pancreatograms and cholangiograms varies considerably and they may be difficult to evaluate. The detection of the subtle changes associated with AIP is enhanced by knowledge and training[23], which was likely to be the case in the revisions of the pancreatologists and research fellows.

With this study, no conclusions can be made regarding the specificity of the diagnostic scoring systems, i.e. false positivity in differential diagnosis with malignancy or chronic pancreatitis. Reports on diagnostic scoring systems generally focus on capability of classifying the presence of disease. With each scoring system, corresponding diagnostic algorithms for differential diagnosis with malignancy were developed[9,10,24,25]. Extensive validation studies of all diagnostic criteria simultaneously are not reported yet. Because of the rarity of the disease, uniformity and international consensus should be pursued. The development of the ICDC was an important step as it combined several good aspects of various scoring systems. They are excellent for research purposes, but in our opinion their clinical use is limited because of their complexity. An international prospective trial, aimed at the optimal diagnostic algorithm for the diagnosis of AIP, is highly desirable.

In conclusion, our data show that at initial clinical presentation, the majority of patients with AIP was correctly identified by any of the three major diagnostic criteria systems, without the need for histology. The systems proved to be complementary rather than overlapping. A small subset of patients did not fulfil the criteria of any of these systems. Though very useful in defining populations and confirming diagnosis of AIP, they should not be regarded as absolute, gold diagnostic standard. In daily practice, we recommend the use of HISORt criteria, and the Asian diagnostic scoring system if a pancreatogram is available (optional) or if IgG4 level is normal (mandatory). If diagnosis is still not confirmed, the ICDC can be used.

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CHAPTER 3

SERUM LEVEL OF CA 19-9 INCREASES ABILITY OF IGG4 TEST TO DISTINGUISH PATIENTS WITH AUTOIMMUNE PANCREATITIS FROM THOSE WITH PANCREATIC CARCINOMA

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ABSTRACT

Background

Autoimmune pancreatitis (AIP) is often difficult to distinguish from pancreatic carcinoma or other pancreatobiliary diseases. High serum levels of carbohydrate antigen 19-9 (Ca 19-9) are indicative of malignancies, whereas high levels of immunoglobulin (Ig)G4 (>1.4 g/L) are characteristic of AIP. We investigated whether serum levels of these proteins can differentiate between these diseases.

Methods

We measured levels of Ca 19-9 and IgG4 in serum samples from 33 patients with AIP, 53 with pancreatic carcinoma, and 145 with other pancreatobiliary disorders. We determined cut-off levels for each assay. Logistic regression analysis was used to evaluate combined data on Ca 19-9, IgG4, and bilirubin levels.

Results

Low levels of Ca 19-9 were independently associated with AIP, compared with pancreatic adenocarcinoma (odds ratio [OR], 0.28; 95% confidence interval [CI], 0.13–0.59; *P*=.0001). Using an upper level of 74 U/ml, the assay for Ca 19-9 identified patients with AIP with 73% sensitivity and 74% specificity. Using a lower level of 2.6 g/L, the assay for IgG4 identified these patients with 70% sensitivity and 100% specificity. Combining data, levels of Ca 19-9 <74 U/ml and IgG4 >1.0 g/L identified patients with AIP with 94% sensitivity and 100% specificity.

Conclusions

Patients with AIP have lower levels of Ca 19-9 than those with pancreatic carcinoma. Measurement of either Ca 19-9 or IgG4 level alone are not accurate for diagnosis. However, the combination of Ca 19-9 <74 U/ml and IgG4 >1.0 g/L distinguish patients with AIP from those with pancreatic carcinoma with 94% sensitivity and 100% specificity.

INTRODUCTION

Autoimmune pancreatitis (AIP) is a rare, distinct condition that often presents with a pancreatic mass, jaundice and weight loss, and thus may mimic pancreatic carcinoma. In a recent study, we described a 2.6% prevalence of AIP in patients undergoing pancreatoduodenectomy for presumed malignancy of the pancreatic head[1]. Biliary involvement is common in AIP, sometimes without overt pancreatic disease, and can be confused with cholangiocarcinoma or primary sclerosing cholangitis (PSC). Frequently AIP represents the pancreatic manifestation of IgG4-related disease, a systemic disorder that may not only involve the pancreas but almost any other organ. AIP can be associated with disorders such as retroperitoneal fibrosis, sialadenitis, prostatitis, interstitial nephritis and inflammatory tumours in lungs, mediastinum or liver. The disease is highly responsive to steroids[2] , a characteristic which can be helpful in establishing the diagnosis[3]. There is no single diagnostic test that reliably differentiates AIP from other disorders. IgG4 is the best single test to distinguish between AIP and malignancy, with an optimal cut-off level of 2.8 g/L (twice the upper limit of normal), yielding sensitivity of 65% and specificity of 98%[4]. Levels up to 2.8 g/L can also be found in patients with pancreatic carcinoma, PSC and other pancreatic disorders. Thereby, the specificity of slightly elevated levels is limited[4-9]. Several other serological markers - total IgG or autoantibodies like antinuclear antibody, rheumatoid factor, anti carbo anhydrase II and antilactoferrin - have been proposed as useful diagnostic tests[10]. However, most of the studies describing the value of these tests lack sufficient validation.

Ca 19-9 is a tumor associated antigen originally isolated from a human colorectal cancer cell line. The level is elevated in the majority of pancreatic carcinoma patients but it lacks diagnostic performance required for early detection or diagnosis due to substantial numbers of false positive and false negative readings[11]. High levels are also observed in other gastrointestinal malignancies including biliary, hepatocellular, colorectal and gastric cancer. A systemic review found an overall sensitivity of 81% and specificity of 90% for pancreatic carcinoma[11]. A value higher than 1000 U/mL usually indicates digestive cancer, with nearly 100% specificity for pancreatic carcinoma[12]. Investigators from India demonstrated that a level above 300 U/ml in mass lesions in chronic pancreatitis was always indicative of malignancy[13]. However, apart from chronic pancreatitis, Ca 19-9 can also be elevated in other benign GI diseases like cirrhosis, primary sclerosing cholangitis, bacterial cholangitis or choledocholithiasis, actually any condition associated with cholestasis[14-16].

In our national AIP cohort, elevated levels of Ca 19-9 were encountered in the majority of patients. Levels above 34 U/ml were found in 58%, above 100 U/ml in 38% and above 300 U/ml in 18% of cases. Several patients even had levels as high as 5000 to 23,000 U/ml[17]. Elevated levels in AIP were also reported in other cohorts[18-23].

In this study, we aimed to evaluate Ca 19-9 levels in AIP, pancreatobiliary malignancies and benign diseases that show clinical similarity with AIP. Secondly we aimed to assess the performance of Ca 19-9 as a diagnostic test to differentiate between AIP and pancreatic carcinoma, single and in combination with IgG4.

METHODS

Patients

Between March 2007 and May 2011 sera were prospectively obtained from consecutive patients presenting in a single, tertiary center with AIP (n=33), pancreatic carcinoma (n=53), cholangiocarcinoma (n=32), chronic pancreatitis (n=52), primary sclerosing cholangitis (n=30) and Sjögren's syndrome (n=31). Sera were obtained with informed consent and were processed immediately, or stored at -80 ˚C. The diagnosis of AIP was made according to the International Consensus Diagnostic Criteria, Asian or HISORT criteria, or a combination of unexplained pancreatic disease, biliary disease/extrapancreatic manifestations and either response to steroids or IgG4-positive serology [17,24-26]. Sera of chronic pancreatitis patients were collected from patients with chronic alcoholic, obstructive or idiopathic pancreatitis. Diagnostic criteria systems of AIP were systematically applied to all patients with chronic pancreatitis to exclude misclassification. Sera of pancreatic carcinoma or cholangiocarcinoma patients were included only if diagnosis was histologically confirmed. PSC and Sjögren's syndrome patients were diagnosed according to accepted criteria[27,28].

Laboratory measurements

Serum Ca 19-9 levels were measured using an electrochemiluminescense immunoassay (ECLIA) on a Modular Analytics E module (Roche Diagnostics Co, Tokyo, Japan). The upper limit provided by the manufacturer was 34 U/ml. IgG4 levels were determined on the Immage 800 Analyzer (Beckman Coulter, Mijdrecht, the Netherlands) using the Peliclass IgG subclass nephelometry kit according to manufacturer's instructions (Sanquin, Amsterdam, the Netherlands). The upper limit of normal provided by the manufacturer was 1.40 g/l. Bilirubin levels were measured using the Diazo method on a Roche modular P analyser (Roche Diagnostics, Almere, The Netherlands). The upper limit of normal provided by the manufacturer was 16 µmol/L. Serum IgG4, bilirubin and Ca19.9 levels were measured simultaneously.

Statistical analysis

Ca 19-9, IgG4 and bilirubin levels were expressed as median value with interquartile range (IQR). One-way analysis and pairwise comparisons with Bonferroni correction for multiple testing were performed to detect significant differences between groups. P values < 0.05 were considered statistically significant. Mann Whitney U test was performed to detect differences in laboratory parameters in subgroups of AIP patients (with or without mass presentation, steroid use and proximal biliary involvement). Logistic regression analysis was applied to study the simultaneous effect of Ca 19-9, IgG4 and bilirubin. Receiver operating characteristic (ROC) analysis was performed to assess optimal cut-off levels. Test characteristics of Ca 19-9, IgG4 and combination were calculated. Statistical analysis was carried out using SPSS Statistics 20.0 Software (IBM, New York, USA).

Medical ethical concerns

The study was approved by the institutional review board of the Erasmus University Medical Center, Rotterdam, the Netherlands.

RESULTS

Ca 19-9 as predictor of AIP

Patient characteristics and laboratory measurements are shown in Table 1. The distribution of Ca 19-9 for the different groups is shown in Figure 1. Ca 19-9 was significantly higher in pancreatic carcinoma (median 349 U/ml, IQR 63 - 1588) and cholangiocarcinoma (median 247 U/ml, IQR 41-2175) than in AIP (median 26 U/ml, IQR 12 - 108), p < 0.001. The level in AIP was significantly higher than in Sjögren's syndrome (median 6 U/ml, IQR 4-15), p=0.009. In AIP, Ca 19-9 levels showed a wide distribution with minimum of 1 U/ml and maximum of 23283 U/ml. Moreover, in PSC markedly elevated levels were also observed (median 59 U/ml, IQR 23-154), minimum 6, maximum 885 U/ml. The median Ca19-9 level in PSC patients however was significantly lower than in patients suffering from cholangiocarcinoma (p=0.009). The unadjusted effect of Ca 19-9 as a predictor of AIP against pancreatic carcinoma showed a strong association of low Ca 19-9 with high probability of AIP (OR=0.42; 95%CI (0.25-0.70), p=0.0002, Table 2). Serum IgG4 was significantly higher in patients with AIP than in all other groups (p<0.001, Table 1). In particular, IgG4 was significantly higher in the AIP group compared to patients with pancreatic carcinoma. Levels of bilirubin did not differ between AIP and malignancy. After adjustment for IgG4 and bilirubin, Ca 19-9 remained an independent predictor of AIP against pancreatic carcinoma (OR=0.28; 95%CI(0.13-0.59), p=0.0001). IgG4 was strongly associated with AIP, while bilirubin was not significantly associated with AIP (all p-values > 0.17, results not shown).

Ca 19-9 in subgroups of AIP patients

In addition to the analysis above, the distribution of Ca 19-9 within specific subgroups of AIP patients was studied. In AIP patients, Ca 19-9 levels were not significantly different between

Table 1. Patient characteristics

	AIP	Pancreatic carcinoma	Cholangio-carcinoma	Chronic pancreatitis	PSC	Sjögren's syndrome	p-overall
Number	33	53	32	52	30	31	
Age (yr)	65 (55-73)	66 (60-71)	66 (57-73)	52 (44-60)	46 (40-53)	*n.a.*	<0.001[1]
Male (n,%)	28 (85%)	27 (51%)	15 (47%)	36 (69%)	23 (77%)	3 (10%)	<0.001[2]
Ca 19-9 (U/ml)	26(12-108)	349(63-1588)*	247(41-2175)*	10(6-24)	59(23-154)	6(4-15)*	<0.001[1]
IgG4 (g/L)	4.7(1.8-10.5)	0.5(0.2-1.1)*	0.6(0.3-1.7)*	0.6(0.3-1.1)*	0.5(0.2-0.9)*	0.2(0.1-0.5)*	<0.001[1]
Bilirubin (µmol/L)	10(8-34)	15(9-57)	23(8-57)	7(5-11)	38(15-109)	0(0-1)	<0.001[1]
Elevated bilirubin (n,%)	12 (36%)	22 (42%)	17 (53%)	5 (10%)	21 (70%)	0 (0%)	<0.001[2]

Age and laboratory tests in median (interquartile range). AIP: autoimmune pancreatitis; PSC: primary sclerosing cholangitis; n.a.: not available.
[1]One way analysis of variance to detect differences between groups, with * p<0.05 between AIP and group (post hoc paired test with correction for multiple testing). [2]Fisher's exact test.

patients with or without mass presentation (p=0.24), steroid use (p=0.88) or proximal biliary involvement (p=0.17) (Table 3). IgG4 and bilirubin levels differed significantly in subgroups with or without steroids (p=0.047 and p=0.002). Furthermore, a sensitivity analysis of Ca 19-9 as predictor for AIP was performed in these subgroups. Results of the unadjusted effects (Ca 19-9 only) as well as the adjusted effects (adjusted for IgG4 and bilirubin) in the different subgroups supported the overall findings.

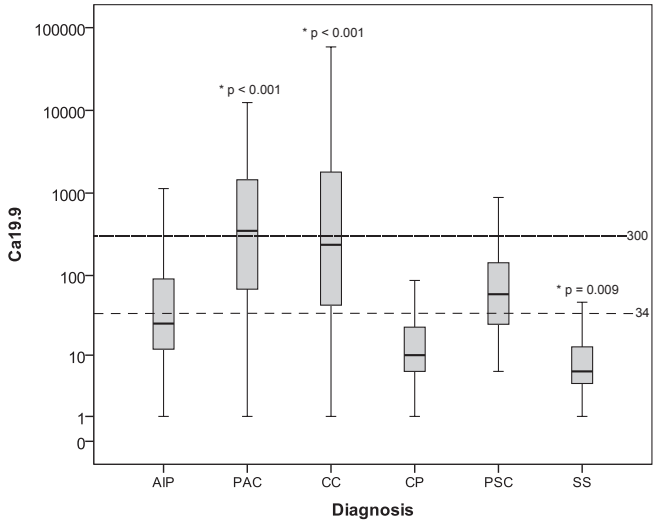

Figure 1. Ca19-9 level in pancreatobiliary diseases and Sjögren's syndrome. Levels of Ca 19-9 are expressed in U/ml, as grey boxes (interquartile range) with median (horizontal line within box) and whiskers (range that contains 95% of observations). AIP: autoimmune pancreatitis; PAC: pancreatic adenocarcinoma; CC: cholangiocarcinoma; CP: chronic pancreatitis; PSC: primary sclerosing cholangitis; SS: Sjögren's syndrome. *p<0.05 between AIP and group.

Table 2. Logistic regression analysis assessing the effect of Ca 19-9, in the total cohort of AIP patients and 3 different subgroups, as predictor for AIP, unadjusted and adjusted for IgG4 and bilirubin

	Unadjusted effect		Adjusted for IgG4 and bilirubin	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Total AIP	0.42 (0.25-0.70)	0.0002	0.28 (0.13-0.59)	0.0001
AIP with mass	0.32 (0.15-0.67)	0.0006	0.07 (0.01-0.51)	<0.0001
AIP steroid naïve	0.50 (0.29-0.85)	0.0066	0.21 (0.07-0.62)	0.0006
AIP proximal biliary involvement excluded	0.41 (0.22-0.78)	0.0030	0.27 (0.11-0.68)	0.0010

Sensitivity analysis (logistic regression analysis) was performed for the total cohort of AIP patients, patients presenting with a mass lesion, those who were steroid naïve and those without proximal biliary involvement. OR: odds ratio; CI: confidence interval.

Table 3. Subgroups of AIP patients: characteristics and relation to Ca 19-9, bilirubin and IgG4

Subgroups		Present	Absent	p-level[1]
Mass	Number, n (%)	12 (38%)	20 (63%)	
	Ca 19-9 (U/ml), median (IQR)	16 (8-56)	34 (12-225)	0.236
	Bilirubin (µmol/l), median (IQR)	9 (7-19)	12 (8-55)	0.224
	IgG4 (g/l), median (IQR)	2.9 (1.4-7.5)	5.0 (2.3-11.7)	0.373
Steroid therapy	Number, n (%)	9 (28 %)	23 (72%)	
	Ca 19-9 (U/ml), median (IQR)	37(13-67)	26 (12-234)	0.881
	Bilirubin (µmol/l), median (IQR)	9 (7-11)	14 (8-61)	0.047*
	IgG4 (g/l), median (IQR)	1.8 (0.7-4.0)	5.4 (3.4-13.6)	0.002*
Proximal biliary involvement[2]	Number, n (%)	13 (50%)	13 (50%)	
	Ca 19-9 (U/ml), median (IQR)	74 (13-251)	25 (6-58)	0.168
	Bilirubin (µmol/l), median (IQR)	20 (10-62)	10 (9-20)	0.264
	IgG4 (g/l), median (IQR)	5.08 (2.6-15.9)	1.89 (1.3-4.9)	0.060

* $p < 0.05$; [1] Mann-Whitney U Test; [2] Biliary involvement other than intrapancreatic part of common bile duct

Diagnostic characteristics of Ca 19-9 and IgG4

The optimal cut-off point for distinction between pancreatic carcinoma and AIP was assessed by ROC analysis, as shown in Figure 2. The area under the curve for Ca 19-9 as predictor for the presence of pancreatic carcinoma was 0.77 (0.66-0.87). The optimal cut-off level was 74 U/ml, yielding a sensitivity of 76% and specificity of 75%, with LR+ of 2.94 and LR- of 0.35. The optimal cut-off for IgG4 was 2.6 g/L, yielding a sensitivity of 70% and specificity of 100%, with LR+ ∞ (infinite) and LR- 0.3.

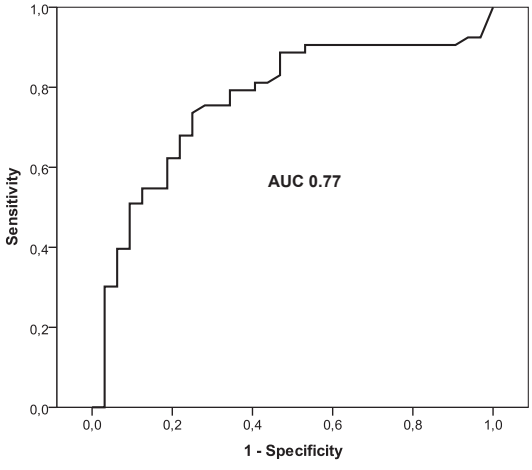

Figure 2. Receiver operating curve for Ca 19-9. The area under the curve (AUC) for the diagnosis of pancreatic carcinoma by means of Ca 19-9 levels was 0.77

The diagnostic performance of Ca 19-9 and IgG4, single and in combination, is shown in Table 4 and Figure 3. Ca 19-9 as a single marker had moderate sensitivity (73%), and specificity

Table 4. Diagnostic performance of Ca 19-9 and IgG4, single and combined, to differentiate AIP from pancreatic carcinoma

	Ca 19-9 ≤ 74	IgG4 > 1.4	IgG4 > 2.6	Ca 19-9 ≤ 74 & 1.0 < IgG4 ≤ 2.6 or IgG4 > 2.6	Ca 19-9 ≤ 74 & 1.4 < IgG4 ≤ 2.6 or IgG4 > 2.6
True positive (n)	24	28	23	29	26
Sensitivity (%)	72.7	84.8	69.7	93.5	83.9
Specificity (%)	73.6	81.1	100	100	100
PPV (%)	63.2	73.7	100	100	100
NPV (%)	81.2	89.6	84.1	96.4	91.4
LR +	2.8	4.5	∞	∞	∞
LR -	0.4	0.2	0.3	0.1	0.2
AUC (95% c.i.)	0.77 (0.66-0.87)	0.91 (0.83-0.99)	0.91 (0.83-0.99)	0.97 (0.92-1.00)	0.92 (0.84-1.00)

PPV: positive predictive value; NPV: negative predictive value; LR: likelihood ratio of + positive or - negative test; AUC: area under the receiver operating curve, CI: confidence interval. Value of Ca 19-9 in U/ml, IgG4 in g/L.

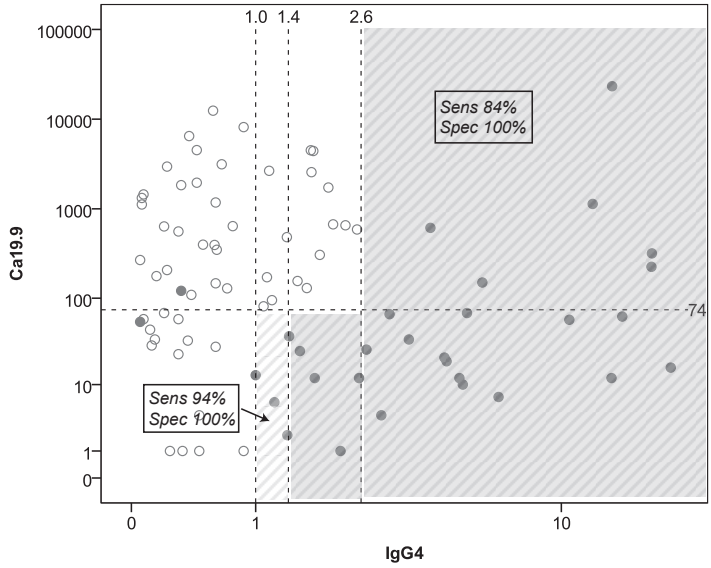

Figure 3. Combination of Ca19-9 and IgG4 to differentiate AIP from pancreatic carcinoma. Circles represent patients with pancreatic carcinoma, dots represent AIP patients. If low levels of Ca 19-9 (<74 U/ml) were combined with IgG4, specificity rose to 100% with a sensitivity of 84% if 1.4<IgG4≤2.6 or IgG4 > 2.6 (grey area) and a sensitivity of 94% if 1.0<IgG4≤2.6 or IgG4>2.6 (diagonally striped area).

(74%). IgG4 levels higher than 1.4 g/L were more sensitive (85%) but moderately specific (81%). Raising the cut-off to 2.6 g/L improved specificity to 100%, at the cost of lowering sensitivity to 70%. However, if low levels of Ca 19-9 (<74 U/ml) were combined with IgG4, specificity rose to 100% and sensitivity improved to 84% if IgG4>1.4 and 94% if IgG4>1.0 g/L.

DISCUSSION

In this study we found that low levels of Ca 19-9 were an independent predictor of AIP after adjustment for IgG4, thus Ca 19-9 appears to provide additional information to distinguish between AIP and pancreatic carcinoma. As a single test, both Ca 19-9 and IgG4 were not optimal for identification of AIP. The most accurate identification of AIP from pancreatic carcinoma was achieved with the combination of low levels of Ca 19-9 (<74 U/ml) and high levels of IgG4 (>1.0 g/L), which shows sensitivity of 94% and specificity of 100%. Our findings hold true for subgroups of AIP patients.

In contrast to previous reports, high levels of Ca 19-9 were not diagnostically helpful. The marked overlap of values in AIP and cancer limit the value of Ca 19-9 in clinical decision making. We observed very high Ca 19-9 levels ranging from 5000-23000 U/ml in several AIP patients, as was reported previously[29]. Previous studies found elevated Ca 19-9 levels in about 25% of AIP cases[18,20,22] as compared to 58% in the present series. In another study addressing the role of Ca 19-9 in differentiating pancreatic carcinoma from AIP, investigators found a sensitivity of 62% and specificity of 92% for values higher than 150 U/ml. The authors concluded that Ca 19-9 levels higher than 150 U/ml are highly specific for pancreatic carcinoma and thus may be useful in differential diagnosis. The different result of the present study may be attributable to differences in patient selection as illustrated by the higher frequency and also higher values of elevated Ca 19-9 in our cohort. This might be explained by differences in proximal biliary involvement, which was more frequent in our AIP patients (50% versus 31-34%[17,18]). However, median levels of bilirubin were not different in patients with or without proximal biliary involvement and bilirubin was not a confounding variable.

In general, the interpretation of elevated Ca 19-9 levels is difficult for several reasons. First, Ca 19-9 is a sialylated Lewis antigen. Seven to 10% of Caucasians (increasing to 22% in Africans) are Lewis negative and are unable to synthesize Ca 19-9[30]. Second, Ca 19-9 can also be elevated in benign pancreatic diseases, which often coexist with pancreatic carcinoma. Third, Ca 19-9 undergoes some degree of biliary excretion and is produced by biliary epithelial cells. Therefore in cholestasis, levels are frequently elevated even in benign conditions[31,32]. Treatment of these conditions may result in normalization[14-16]. In our study, low Ca 19-9 was a strong predictor of AIP, even after adjustment for bilirubin.

For the clinically highly relevant question how to differentiate pancreatic carcinoma from AIP, a test with very high sensitivity and specificity is needed. In this patient group however, the importance of a high sensitivity outweighs a high specificity. A positive test may prompt the decision to perform surgery. In this case clinicians are more willing to accept false positivity (lower specificity) than false negativity (lower sensitivity). The former will lead to a resection with postoperative benign histology (which is generally observed in 5-11% of

pancreatoduodenectomies [1,33-38]), the latter means that patients are deprived of the only chance for cure in the case of true carcinoma.

As single tests to detect AIP, Ca 19-9 (<74 U/ml) and IgG4 (>2.6 g/L) would entail 27% and 30% of AIP patients misclassified as cancer while having AIP (sensitivity 73% and 70%) respectively. This means that one quarter to one third of patients undergoing resection would be erroneously exposed to a surgical procedure with substantial morbidity (46%) and mortality (though in a large volume centers less than 5%) [35,39]. Ca 19-9<74 U.ml as a single test corresponds with 26% false positive rate (specificity of 74%), that means one quarter of patients with cancer would be erroneously treated with prednisone and potentially deprived of a curative surgical resection. IgG4 levels of >2.6 g/L are 100% specific, thus raising the normal cut-off (1.4 g/L) to this level would protect AIP patients from unnecessary surgery. For comparison, in large cohorts of AIP patients, 16-30% underwent resection[17,20,22,24,40]. This optimal cut-off level of IgG4 is in line with previous observations, which suggested a level of 2.8 g/L (twice the upper limit of normal)[4,6]. In our study, IgG4 levels higher than 1.4 g/L were fairly sensitive for AIP (85%) but not very specific (81%). Interestingly, if low levels of Ca 19-9 (<74 U/ml) were combined with IgG4, specificity rose to 100% and sensitivity improved to 84% if IgG4>1.4 and 94% if IgG4>1.0. Thus, low levels of Ca 19-9 improve both the moderate specificity of intermediate IgG4 levels, as the poor sensitivity of high IgG4 levels.

To determine the quality of a diagnostic test in clinical practice, most studies report predictive values. In diseases with low prevalence however, this might cause overestimation of the quality of the test. The likelihood ratio of positive (LR+) and negative result (LR-), are the stable characteristics of a test, independent of prevalence[41]. In our study, LR+ of IgG4 > 2.6, or intermediate levels of IgG4 in combination with Ca 19-9 < 74 U/ml, were infinite. That means that these levels definitely rule in diagnosis of AIP. Values of these markers outside this range have less capacity to rule out AIP as reflected by their LR-.

Although the use of Ca 19-9 is advocated in several diagnostic algorithms for AIP, as far as we are aware, this is the first study addressing the combination of both markers to improve the diagnostic power in discrimination between AIP and pancreatic cancer. The strengths of our study include the diversity of the control groups and the careful selection of patients. Malignancy was always confirmed with histology, and chronic pancreatitis patients were excluded if they scored positive on diagnostic criteria systems for AIP. Furthermore, we believe that expressing the value of this test in terms of likelihood ratios gives a more accurate reflection of its use in daily practice than the commonly reported sensitivity, specificity and predictive values.

Two possible sources of misclassification bias are present in this study. For diagnosing AIP we used generally accepted diagnostic criteria systems as gold standard. This might, but does not always, include histological proof. In theory, some of these patients could have been wrongly classified as AIP while having malignancy. However, all patients in our cohort were observed at least two years, which virtually rules out cancer. In patients with cholangiocarcinoma, several patients with underlying PSC were included. We cannot rule out the possibility of occult malignancy in PSC patients. Our study was not designed to address this particular issue. This renders any conclusion regarding the Ca19-9 values in PSC in relation to cholangiocarcinoma less reliable.

In conclusion, the present study showed that low levels of Ca 19-9 independently predict AIP against pancreatic carcinoma. Both Ca 19-9 and IgG4 are not optimal as single markers. The most accurate identification of AIP patients was achieved with the combination of Ca 19-9 (<74 U/ml) and IgG4 (>1.0 g/L), which showed sensitivity of 94% and specificity of 100%.

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CHAPTER 4

THE VALUE OF ELEVATED SERUM IGG4 AND IGG4/IGG2 RATIO IN AUTOIMMUNE, ACUTE AND CHRONIC PANCREATITIS

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Submitted

ABSTRACT

Background

Type 1 autoimmune pancreatitis (AIP) is the pancreatic manifestation of a systemic IgG4-related fibroinflammatory disorder. Differentiating AIP from other forms of acute pancreatitis (AP) and chronic pancreatitis (CP) can be challenging. We evaluated the prevalence of elevated levels of total IgG, its subclasses, and IgE in patients with type 1 AIP, AP and CP.

Methods

Sera of patients with AIP and CP were obtained between March 2007 and May 2011, from consecutive cases presented to our tertiary referral center. Sera from patients with (AP) were derived from a tissue bank, created between March 2004 and March 2007. In all samples, IgG, its subclasses, and IgE levels were determined.

Results

A total of 174 patients were included; 32 AIP, 90 AP, and 52 CP patients. Elevated IgG4 levels (upper limit of normal [ULN] 1.4 g/L) were found in 27 AIP patients (84%), but also in 7 AP (8%) and 9 CP (17%) patients ($p<0.001$). IgG4 levels >2 times the ULN were found in 19 AIP patients (63%), in nil AP and 3 CP patients (6%; $p<0.001$). In patients with a serum IgG4 between 1 and 2 times the ULN, the PPV was just 12%. In this subgroup, applying the IgG4/IgG2 ratio improved the PPV from 12 to 75%.

Conclusions

Elevated serum IgG4 levels are frequently present in patients with AP and CP, and therefore must be interpreted with caution. However, levels above twice the upper limit of normal are rare in AP and CP, and suggest AIP. In mildly elevated IgG4 (1-2x ULN), the IgG4/IgG2 ratio can substantially improve the positive predictive value.

INTRODUCTION

Autoimmune pancreatitis (AIP) is a rare form of pancreatitis that responds dramatically to steroid therapy and typically affects men older than 55 years [1]. Two subtypes are distinguished [2,3]. Type 1 represents the pancreatic manifestation of IgG4-related systemic disease (IgG4-RD) and may involve other organs, such as the biliary tree, kidneys, and salivary glands [4]. Type 2 is rare, pancreas-specific, and not associated with IgG4 [5].

AIP patients generally present with obstructive jaundice, weight loss, and diabetes. Therefore, the primary diagnostic consideration is often pancreatic cancer. In addition, it can also be challenging to distinguish AIP from other types of pancreatitis. Sah et al. reported that 24% of AIP patients have features of acute pancreatitis (AP); severe abdominal pain, elevated lipase levels, or an oedematous pancreas with peripancreatic stranding [6]. Also, in a more advanced stage, AIP may have characteristics of chronic pancreatitis (CP), such as pancreatic atrophy, ductal stricturing, and functional impairment [7,8].

IgG4, a subclass of the immunoglobulin G (IgG), is often used as a diagnostic tool in this setting. It accounts for less than 5% of total serum IgG and, although the normal value varies, it is stable in healthy individuals [9-11]. In 2001, elevated serum IgG4 levels were first observed in AIP patients [12]. Nowadays, it is the mainstay of establishing the diagnosis of type 1 AIP [13,14].

However, particular caution is needed in interpreting elevated levels of IgG4, since they are also encountered in other disorders, including chronic pancreatitis and primary sclerosing cholangitis [12,15,16]. Very little data are available regarding IgG4 levels in AP of non-immune aetiology. Previous studies have shown that combined serum IgG4 and IgG1 measurement can be helpful to distinguish IgG4-associated cholangitis from primary sclerosing cholangitis [17]. So far, combining different IgG subclasses to differentiate AIP from AP and CP has not been investigated. Furthermore, IgE may also be of value, as several studies have reported this immunoglobulin to be elevated in AIP [18-20]. Therefore, the aim of this study was to evaluate serum levels of IgG, its subclasses, and IgE in patients with either type 1 AIP or acute and chronic pancreatitis of non-immune aetiology.

METHODS

Patients

The study was approved by the Institutional Review Board of the Erasmus University Medical Center. For AIP and CP, serum samples were obtained (after written informed consent) from consecutive patients, presenting to our tertiary referral center between March 2007 and May 2011. Sera from patients with acute biliary, alcoholic or idiopathic pancreatitis were derived from a series of 732 samples that had been collected between March 2000 and March 2007, in the course of a prospective trial that ran within the Dutch Pancreatitis Study Group [21]. Samples were matched for age and gender.

To allow a diagnosis of AIP, patients had to fulfil either the HISORt criteria (Histology, Imaging, Serology, Other organ involvement, and Response to steroid therapy) or the International Consensus Diagnostic Criteria (ICDC) [13,14]. AP was defined as abdominal pain with an at least three-fold elevation of serum amylase or lipase levels. CP was diagnosed, based on

clinical symptoms, combined with morphological features (calcifications and ductal changes) and/or pancreatic functional insufficiency.

Laboratory analysis and normal values

Serum samples were either processed immediately or stored at -80°C. IgG levels were determined by routine automated turbidimetry with a Cobas 8000 modular analyser (Roche, Almere, The Netherlands). The upper limit of normal, provided by the manufacturer, is 16.0 g/L. IgG subclasses were determined using the Peliclass IgG subclass nephelometry kit (Sanquin, Amsterdam, The Netherlands), with an Immage 800 Analyzer (Beckman Coulter, Mijdrecht, The Netherlands). The upper limits of normal of IgG1 to 4 are; 11.4, 6.4, 1.1, and 1.4 g/L, respectively. Total IgE was determined by the ImmunoCAP 250 system (Phadia, Nieuwegein, The Netherlands). For IgE, no upper limit of normal is defined.

Statistical analysis

Data were analyzed using SPSS Statistics 20.0 Software (IBM, New York, USA). Serum levels are presented as median values with interquartile ranges (IQR). To detect differences between groups, the Mann-Whitney U Test (for continuous data) or the Fisher's Exact Test (for categorical data) were used. The Kruskal-Wallis test was used to detect differences between the three groups. Furthermore, IgG4/subclass ratios were determined. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and receiver operating characteristic (ROC) curves were used to assess the diagnostic utility of serum IgG, IgG subclasses, IgG4/subclass ratios, and IgE in AIP. Logistic regression analysis was performed to study the differences in occurrence of elevated levels of IgG, IgG1, IgG4, IgG3, and IgE between the three groups. P-values <0.05 were considered to be statistically significant.

RESULTS

Patients

In total, samples from 174 patients were evaluated; 32 with AIP, 90 with AP and 52 with CP. Patient and disease characteristics are shown in Table 1. Among the AIP patients, 24 (75%) presented with obstructive jaundice, 29 (91%) with weight loss, and 17 (53%) with (mild) abdominal pain. Other organs were involved in 23 patients (72%), most frequently extra-pancreatic cholangitis (19 patients, 61%). In eight patients (25%), more than one extra-pancreatic manifestation was observed. AIP patients were older and more often male, as compared to AP and CP patients ($p<0.001$). Nine AIP patients were receiving steroid treatment at the time of blood sampling (28%).

IgG4 levels

Elevated IgG4 levels were observed in 25 (75%) of the AIP patients, but also in seven (8%) AP patients and in nine (17%) CP patients (Table 2, Figure 1). However, compared with AIP, AP and CP patients were less likely to have serum IgG4 levels more than twice the upper limit of normal (ULN) (63% vs 0%, $p<0.001$; 63% vs 6%, $p<0.001$).

Other immunoglobulin levels

Univariate analysis revealed that elevated levels of total IgG and IgG1 were more prevalent in the AIP group, as compared to the AP group (p<0.001 and p=0.001, respectively). The same conclusion was drawn for IgG, IgG1 and IgG3 for AIP versus CP patients (p<0.001, p<0.001, p=0.007, respectively).

Furthermore, IgE was also significantly higher in AIP patients (p<0.001 and p=0.001; Table 2, Figure 2). However, none of these markers had a better diagnostic performance than serum IgG4 (Table 3). There were no significant differences in immunoglobulin levels between AP and CP patients and, therefore, in further analyses, the results for AP and CP patients were combined.

Table 1. Demographics of patients with AIP, AP, and CP.

Characteristic	AIP (n=32)	AP (n=90)	CP (n=52)	p-value
Age in years[¥] - median (IQR)	66 (54-73)	56 (43-69)	52 (44-60)	0.003[1,*]
Male sex – no. (%)	28 (88)	45 (50)	36 (69)	<0.001[1,*]
Disease specifics – no. (%)	**Location** Diffuse: 20 (65) Focal: 6 (19)	**Cause** Alcoholic: 28 (31) Idiopathic: 30 (33) Biliary: 32 (36)	**Cause** Alcoholic: 29 (56) Idiopathic: 14 (27) Other[¥]: 9 (17)	-

[¥] Age when sample was taken; * p < 0.05; [1]P-value corresponds with differences between the 3 groups; [1]Kruskal-Wallis Test; [2]Fisher's Exact Test
AIP = autoimmune pancreatitis; CP = chronic pancreatitis; AP = acute pancreatitis; IQR = inter quartile range.
[¥]Other causes of chronic pancreatitis: pancreas divisum, iatrogenic, hereditary.

Table 2. Serum IgG, IgG subclasses and IgE in patients with AIP, AP, and CP.

Characteristic	AIP (n=32)	AP (n=90)	CP (n=52)	AIP vs AP p-value	AIP vs CP p-value
Total IgG – g/L[¶]	12.7 (10.5-21.7)	9.5 (7.7-11.1)	10.5 (8.9-12.6)	< 0.001[1,*]	0.005[1,*]
>16.0 – no. (%)	13 (41)	3 (3)	1 (2)	< 0.001[2,*]	< 0.001[2,*]
IgG4 – g/L[¶]	4.5 (1.7-7.7)	0.4 (0.2-0.9)	0.6 (0.3-1.1)	< 0.001[1,*]	< 0.001[1,*]
range	0.1-17.2	0.1-1.85	0.1-4.1		
>1.4 – no. (%)	25 (83)	7 (8)	9 (17)	< 0.001[2,*]	< 0.001[2,*]
>2.8 – no. (%)	19 (63)	0 (0)	3 (6)	< 0.001[2,*]	< 0.001[2,*]
IgG1 – g/L[¶]	7.7 (5.9-11.9)	5.4 (4.3-6.9)	6.2 (5.3-7.9)	< 0.001[1,*]	0.028[1]
>11.4 – no. (%)	8 (25)	3 (3)	1 (2)	0.001[2,*]	< 0.001[2,*]
IgG2 – g/L[¶]	3.1 (2.1-5.1)	2.9 (2.1-4.3)	3.5 (2.6-5.1)	0.505[1]	0.407[1]
>6.4 – no. (%)	2 (6)	5 (6)	6 (12)	0.999[2]	0.704[2]
IgG3 – g/L[¶]	0.6 (0.4-1.0)	0.4 (0.3-0.7)	0.4 (0.3-0.6)	0.025[1]	0.001[1,*]
>1.1 – no. (%)	5 (16)	5 (6)	0 (0)	0.126[2]	0.007[2,*]
IgE – g/L[¶]	302 (50-978)	38 (16-104)	47 (19-102)	< 0.001[1,*]	0.001[1,*]
>100 – no. (%)	15 (65)	23 (26)	13 (26)	0.001[2,*]	0.002[2,*]

[¶]Given values are medians with inter quartile range (IQR); *p < 0.05; [1] Mann-Whitney U Test; [2] Fisher's Exact Test
The upper limits of normal (ULN) were 16.0 g/L (IgG), 11.40 (IgG1), 6.40 (IgG2), 1.10 (IgG3) and 1.40 (IgG4) g/L. For IgE, no ULN is available. AIP = autoimmune pancreatitis; CP = chronic pancreatitis; AP = acute pancreatitis.

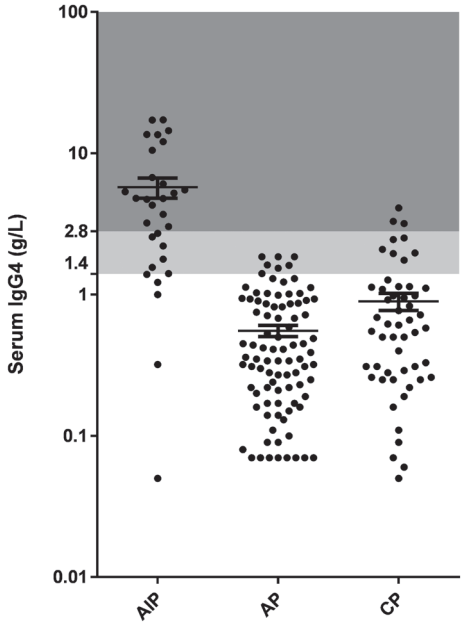

Figure 1. Scatterplot of serum IgG4 in AIP, AP and CP patients. The upper limit of normal (ULN) and 2xULN are represented by different shades of grey. The bar across each column represents the median value.

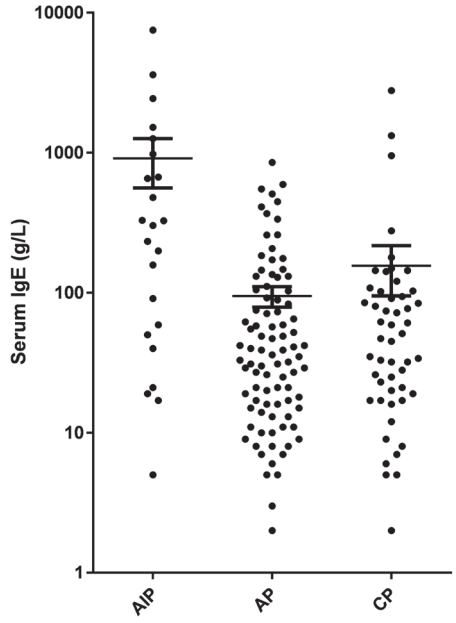

Figure 2. Boxplots of serum IgE in acute, chronic and autoimmune pancreatitis.

IgG4 cut-off value

The discriminative value of IgG4 varied according to the cut-off value. At a value of 1.40 mg/dL (ULN), the sensitivity and specificity were 83% and 89%, respectively (Table 3). The matching PPV was 61% and the NPV 96%. Raising the cut-off value to 2.8 mg/dL (twice the ULN), decreased the sensitivity to 63%, but improved the specificity to 98%. The PPV increased substantially to 86%, whereas the NPV hardly fell (from 96% to 93%). Raising the cut-off value even further, to 4x the ULN, resulted in a drastic decline in sensitivity, down to 30% (95% CI: 14-46).

IgG4/subclass ratios

Interestingly, when comparing IgG4/subclass ratios between AIP and non-AIP patients, all ratios were higher in AIP patients (Table 4). The IgG4/IgG2 ratio showed the highest area under the curve (AUC). At a cut-off value of 0.97, it harbored the optimal combination of sensitivity (76%; 95% CI: 59-93) and specificity (88%; 71-100), with a PPV of 90% (79-100) and a NPV of 70% (50-90) (Figure 3).

Table 3. Univariate performance of Immunoglobulins IgG, IgG subclasses and IgE in distinguishing autoimmune pancreatitis from acute pancreatitis and chronic pancreatitis.

	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
IgG4 > 1.4 g/L	83 (70-97)	89 (84-94)	61 (45-77)	96 (93-100)
IgG4 > 2.8 g/L	63 (46-81)	98 (96-100)	86 (71-100)	93 (88-97)
IgG4 > 5.6 g/L	30 (14-46)	100 (100-100)	100 (100-100)	87 (82-92)
Total IgG > 16 g/L	41 (24-58)	97 (95-100)	77 (54-99)	88 (83-93)
IgG1 > 11.4 g/L	25 (10-40)	97 (95-100)	67 (35-98)	85 (80-91)
IgG2 > 6.4 g/L	6 (-2-15)	92 (88-97)	15 (38-100)	81 (75-87)
IgG3 > 1.1 g/L	16 (3-28)	97 (93-100)	50 (12-88)	84 (78-89)
IgE > 100 g/L	67 (48-86)	57 (51-63)	29 (16-42)	93 (88-98)

The upper limits of normal (ULN); 16.0 g/L (IgG), 11.40 (IgG1), 6.40 (IgG2), 1.10 (IgG3), and 1.40 (IgG4). For IgE, no ULN is available.

Table 4. Serum IgG4 ratio analysis in patients with elevated IgG4.

Subclass ratio, median (IQR)	AIP	Non-AIP; AP + CP	p-value	AUC (95% CI)
IgG4/**IgG**	0.30 (0.26-0.53)	0.17 (0.15-0.20)	0.001	0.81 (0.67-0.95)
IgG4/**IgG1**	0.60 (0.41-0.92)	0.29 (0.23-0.37)	0.001	0.82 (0.68-0.95)
IgG4/**IgG2**	1.75 (0.94-2.65)	0.53 (0.33-0.91)	<0.001	0.85 (0.73-0.97)
IgG4/**IgG3**	8.27 (4.85-10.34)	4.04 (2.81-8.03)	0.035	0.70 (0.52-0.88)
IgG4/**IgE**	0.02 (0.01-0.08)	0.03 (0.01-0.07)	0.730	0.47 (0.27-0.66)

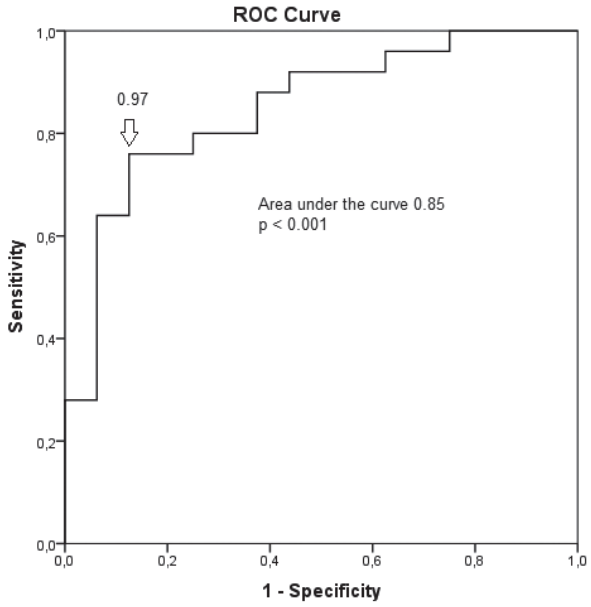

Figure 3. ROC curve evaluating diagnostic value of IgG4/IgG2 ratio in AIP versus AP and CP with elevated IgG4.

Our previously described results, showed that most non-AIP patients with elevated IgG4 had values in the range of 1.4 to 2.8 (Figure 1), which correspond with a PPV of only 12%. When the IgG4/IgG2 ratio was applied, the PPV for this group improved to 75% (95% CI: 33-100), with a NPV of 80% (95% CI: 60-100).

DISCUSSION

Our results show that elevated IgG4 levels occur in almost one out of ten AP patients and one out of five CP patients. However, in AP and CP, IgG4 levels rarely exceed twice the upper limit of normal. Thus, the PPV for AIP in patients with mildly elevated IgG4 is rather low, but can be substantially improved by applying the IgG4/IgG2 ratio.

In line with our findings, previous studies have shown that elevated serum IgG4 does not preclude other forms of pancreatitis [15,16]. In the subgroup with mildly elevated IgG4, the risk of misdiagnosis and mistreatment is apparent. However, a markedly elevated IgG4 is a reliable marker for AIP. Our study, with a large number of individuals from different, well-characterized patient populations, confirm earlier reports, to raise the cut-off value to twice the ULN to improve the diagnostic value [16,22].

We found that none of the other IgG subclasses could compete with IgG4 as individual predictor of AIP. However, applying the IgG4/IgG2 ratio has additional benefit. A previous study found that the IgG4/IgG1 ratio improved the ability to differentiate IgG4-associated cholangitis

from primary sclerosing cholangitis [17]. In our cohort, the IgG4/IgG1 ratio was also higher in AIP patients, but the diagnostic performance of the IgG4/IgG2 ratio was slightly superior.

For cholangitis, these differences in IgG4 ratios were explained by the fact that 23-30% of the primary sclerosing cholangitis patients showed generalized hypergammaglobulinemia, with elevation of all IgG subtypes, and may be induced by a nonspecific activation of the immune system [17,23]. In contrast, IgG4-associated cholangitis patients had an isolated elevation of IgG4, leading to elevated IgG4 ratios. However, this explanation does not apply for our cohort of AP and CP patients, as they did not have hypergammaglobulinemia. Elevated ratios in our AIP patients might therefore be solely explained by the marked elevation of IgG4 in AIP patients, compared to the usually mild elevation in AP and CP. The use of serum IgG4/subclass ratios in distinguishing AIP from AP and CP has not been described before.

Boonstra et al designed a diagnostic algorithm to differentiate between the various bile duct disorders, which was evaluated in a validation cohort. In analogy, the diagnostic algorithm depicted in figure 4 is based on the current data. With IgG4 testing alone, 16 AP and CP patients would have been misclassified as AIP. With the proposed algorithm, 14 of these patients (88%) would have been diagnosed correctly. Obviously, this algorithm requires validation in other patient cohorts.

We also found IgE levels to be higher in AIP, compared to AP and CP patients. This is not an uncommon finding, as both IgG4 and IgE are considered to be part of an Th2 immune response [9]. Furthermore, elevated IgE levels were previously reported in patients with IgG4-related tubulointerstitial nephritis [24]. Also, AIP patients showed significantly higher IgE levels, compared to pancreatic cancer patients, in a pilot study [18]. A larger study should confirm whether this is a true predictor of AIP.

The pathophysiology of AIP and IgG4-RD is still unclear, and both autoimmune and allergic responses have been postulated as underlying mechanisms [25,26]. Elevated IgE levels, which have been described before, support the theory of an allergic response [18,27]. Recently, an autoimmune response to occupational antigen exposure has been suggested to play a role [28]. Chronic antigen stimulation by solvents, oils, or industrial dusts during a career in a blue-collar profession, similar to reported elevated IgG4 serum levels in beekeepers [29], is hypothesized to be a potential trigger of IgG4-related disease in susceptible individuals.

Our study is the first to investigate combined immunoglobulin testing in a large group of AIP, AP and CP patients. Furthermore, we evaluated the value of IgG4/IgG subclass ratios, to provide more reliable differential diagnostic tools. However, there are several limitations

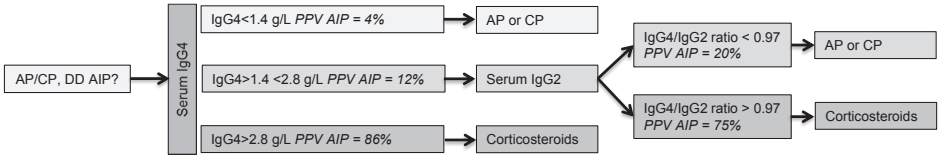

Figure 4. Diagnostic algorithm for distinguishing AIP from AP or CP using serum IgG4 and IgG4/IgG2 ratio analysis.

to our study. For one, we did not possess the long-term clinical outcome data of all patients. In addition, serum samples were not obtained at the same time in the disease course (e.g. at presentation, when a patient first developed symptoms) and some AIP patients already had had steroids.

In conclusion, we showed that mildly elevated IgG4 levels not only occur in AIP, but also in AP and CP patients and must therefore be interpreted with caution. However, IgG4 levels above twice the upper limit of normal do not occur in AP patients and are rare in CP patients. Therefore, to establish a diagnosis of AIP, increasing the IgG4 cut-off value seems inevitable. We also recommend combining IgG4 with IgG2 testing, to improve diagnostic accuracy.

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CHAPTER 5

TESTING FOR ANTI-PBP ANTIBODY IS NOT USEFUL IN DIAGNOSING AUTOIMMUNE PANCREATITIS

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Submitted

ABSTRACT

Objectives

Autoimmune pancreatitis (AIP) is a rare form of chronic pancreatitis, clinically mimicking pancreatic cancer. In 2009, a serological marker for AIP, anti-PBP antibodies, was identified, with an outstanding sensitivity and specificity (NEJM 361:135). We aimed to validate the usefulness of serum antibodies against AKEERRY in identifying patients with type 1 AIP.

Methods

Between March 2007 and May 2011 sera were collected from consecutive patients presenting with type 1 AIP, pancreatic adenocarcinoma (PAC), chronic pancreatitis (CP), primary sclerosing cholangitis (PSC), and healthy controls (HC) with or without antibodies against *Helicobacter pylori*. Serum antibody binding to synthetic PBP peptide was quantified by enzyme-linked immunosorbent assay (ELISA), using standard curves of custom-made PBP rabbit polyclonal antibodies. A synthetic FLAG-peptide (DYKDDDK), to which no antibodies are found in human serum, was included as negative control.

Results

High sensitivity of AKEERRY peptide recognition was demonstrated by selective binding of PBP peptide over Flag-peptide by PBP-immunized rabbit serum. Competition assays with PBP peptide validated the selectivity for antibodies recognizing this antigen. A total of 114 patients were subsequently tested; 34 AIP, 29 PAC, 17 CP, 16 PSC, and 18 HC's (9 positive and 9 negative for H. pylori). No significant differences in detection of antibodies against the PBP peptide were found between different patient groups and healthy controls.

Conclusions

Using a sensitive and selective ELISA-based assay, we did not find increased serum antibodies against the PBP peptide AKEERRY in AIP patients. PBP serum antibodies are therefore not a useful diagnostic tool to diagnose AIP.

INTRODUCTION

Autoimmune pancreatitis (AIP) is a distinct type of pancreatitis, often part of a systemic IgG4-related disease (IgG4-RD). This fibro-inflammatory disorder may also involve other organs, such as the biliary tree, kidneys, and salivary glands [1]. Unlike other types of pancreatitis, AIP responds extremely well to steroid therapy [2]. Clinically, AIP may mimic pancreatic cancer (PAC), with jaundice and weight loss as most common symptoms, and differentiation can be difficult.

As serum IgG4 lacks diagnostic accuracy with a sensitivity of 76% and specificity of 93%, a number of other serological markers have been proposed [3-16]. However, most were reported in a single publication only and none could be validated in a Western population [17].

In 2009, Frulloni *et al* identified a novel serological marker by screening a random peptide library with pooled IgG obtained from 20 AIP patients [18]. The identified peptide showed high homology with plasminogen-binding protein (PBP) from *Helicobacter pylori* as well as the human ubiquitin-protein ligase E3 component n-recognin 2 (UBR2), which is expressed in pancreatic acinar cells. In 94% of AIP patients and only 5% of PAC patients, IgG antibodies against PBP peptide (amino acid sequence AKEERRY) were subsequently observed, resulting in a diagnostic tool with an outstanding sensitivity and specificity of 94% and 95%, respectively. However, the promising results of this study have not been confirmed.

The aim of the present study was to validate the usefulness of serum IgG antibodies against PBP peptide in discriminating type 1 AIP from PAC, chronic pancreatitis (CP), and primary sclerosing cholangitis (PSC).

METHODS

Patients

Between March 2007 and May 2011, sera were obtained from consecutive patients presenting with AIP, pancreatic carcinoma (PAC), chronic pancreatitis (CP) and primary sclerosing cholangitis (PSC). Furthermore, samples of healthy controls, with and without antibodies against *H.pylori*, were collected. Sera were stored at -80°C until use. The study was approved by the Institutional Review Board of the Erasmus MC University Medical Center (MEC-2013-581).

Baseline characteristics of the different patient groups are summarized in Table 1. Patients with AIP were included if they fulfilled the ICDC or HISORt diagnostic criteria for AIP[19,20]. All AIP patients were of type 1 and 26 had extrapancreatic manifestations (77%). Twenty-four of the samples were collected at diagnosis (71%) and 10 after initiation of steroid treatment (29%). To prevent misdiagnosis, any chronic pancreatitis patients that fulfilled the AIP criteria were excluded.

All cases with pancreatic cancer were histologically proven and PSC patients were diagnosed according to accepted criteria [21]. Serum samples of healthy controls were obtained from randomly selected blood donors and evaluated for *H.pylori* specific antibodies by a commercial ELISA kit (Pyloriset EIA-G-III, Orion diagnostics, Renkum, the Netherlands). At a positive cut off titre of ≥20, this test achieved a sensitivity of 100% (95% CI 95.6-100%) and a specificity of 94,3% (95% CI 88.6-97.7%).

Table 1. Baseline characteristics of all included patients (n=114)

Diagnosis	No. of patients	Median age (IQR), y	Male gender, no. (%)
Autoimmune pancreatitis	34	66 (56-72)	29 (85)
Pancreatic carcinoma	29	65 (61-70)	17 (59)
Chronic pancreatitis	17	57 (49-62)	10 (59)
Primary sclerosing cholangitis	16	46 (39-54)	12 (75)
Healthy controls: *H.pylori* - serological status	9	62 (62-65)	6 (67)
Healthy controls: *H.pylori* + serological status	9	63 (59-65)	7 (78)

Peptide synthesis

The amino acid sequence of plasminogen-binding protein (PBP) of *Helicobacter pylori*, as described by Frulloni et al [18] (AKEERRY), was manually synthesized with the standard method of Fmoc solid-phase peptide synthesis (Mimotopes, Melbourne, Australia). Total peptide sequence was Biotin-SGSGAKEERRY-NH2. Biotin was included to facilitate binding of the peptide to neutravidin-coated immunoplates (Pierce, Thermo Fisher Scientific, Rockford, IL.), as we observed low binding of unbiotinilated peptide to uncoated plates in standard carbonate buffer; and to ensure equal binding of peptide (at saturating levels) in all wells. The linker sequence was added as per manufacturers' recommendation.

Initial experiments using a variety of plastics and blocking agents showed high background levels, due to aspecific binding of human immunoglobulins. We therefore included a synthetic control peptide (FLAG-peptide, sequence Biotin-SGSGDYKDDDDK-NH2) to which no natural antibodies occur in human serum, which served as negative control.

Rabbit antiserum production

Custom polyclonal rabbit antibodies against the AKEERRY sequence were generated by Eurogentec (Maastricht, the Netherlands), using the Speedy mini protocol. As small peptides are generally less immunogenic, the immunisation peptide used was AKEERRYAKEERRY. *In silico* analysis showed that this peptide was highly immunogenic across the whole sequence, and post-immunization serum specifically recognized the AKEERRY sequence.

Assessment of antibody binding

Antibody binding was evaluated by means of a modified enzyme-linked immunosorbent assay (ELISA). Pre-blocked Neutravidin-coated plates (Thermo Fisher Scientific, Rockford, IL) were coated with 10 µl/ml peptide in phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin (BSA), resulting in peptide binding at saturating levels (not shown). Plates were incubated at room temperature for one hour and unbound peptide was removed by washing four times with wash-buffer (PBS with 0.05% Tween-20). Plates were incubated overnight at 4 ºC with serum samples, diluted 1:50 in PBS/2% BSA. Plates were washed four times with

wash-buffer and incubated with peroxidase-labeled antihuman IgG antiserum (Jackson ImmunoResearch, 1:1600 in PBS/2% BSA). Plates were then washed five times with wash-buffer, followed by incubation with 3,3',5,5'-Tetramethylbenzidine (TMB) substrate solution (eBioscience). The reaction was stopped by addition of 2N H2SO4 and plates were read on a Bio-Rad 680 XR microplate reader. The data were analysed with the software provided by the manufacturer.

In each individual experiment, standard curves were performed using serial dilutions of custom-made rabbit polyclonal antibodies against PBP. Each serum sample was tested in triplicate for reactivity against AKEERRY peptide and the non-physiological FLAG-peptide. The latter values were subtracted as negative control. Anti-Flag antibodies (Signalway antibody, College Park, MD) were used to confirm saturated binding of the Flag-peptide.

Statistical analysis

Statistical analyses were performed using Graphpad Prism 5.0 and SPSS Statistics 20.0 Software. The nonparametric Kruskal-Wallis test was used to detect differences between groups. P-values <0.05 were considered to be statistically significant.

RESULTS

In order to test whether the presence of antibodies against the *H.pylori* PBP peptide could distinguish between AIP and PAC patients, we set up an ELISA assay, which sensitively and reliably measures reactivity against the AKEERRY peptide sequence in serum. Rabbit immunization with a tandem repeat of the AKEERRY protein resulted in the production of rabbit polyclonal antibodies specifically detecting the PBP, but not Flag-peptide by ELISA (Figure 1A). Serial dilutions showed that PBP peptide could be reliably measured above background, with serum diluted up to 50,000 times (Figure 1B), equivalent with purified antibodies up to 20 ng/mL. To further demonstrate specificity of the assay, we performed a competition assay, which showed selective inhibition of serum antibody binding to the helicobacter peptide, when serum was pre-mixed with PBP peptide, but not when Flag peptide was added (Figure 1C).

Patients

Having optimized our assay to detect serum PBP antibodies, we subsequently tested a total of 114 patients; 34 patients with AIP, 29 with PAC, 17 with CP, 16 with PSC, 9 *H.pylori* negative healthy controls, and 9 *H.pylori* positive healthy controls. Considerable variation in antibody binding was observed between patients (examples shown in Figure 2A). However, no significant difference in detection of antibodies against the PBP peptide was found among the different patient groups or healthy controls (Figure 2B). In addition, sera from *H. pylori* positive controls did not show higher reactivity towards the PBP peptide than *H. pylori* negative sera (73.05±33.47 vs 38.85±12.67, p=0.18; Figure 2B).

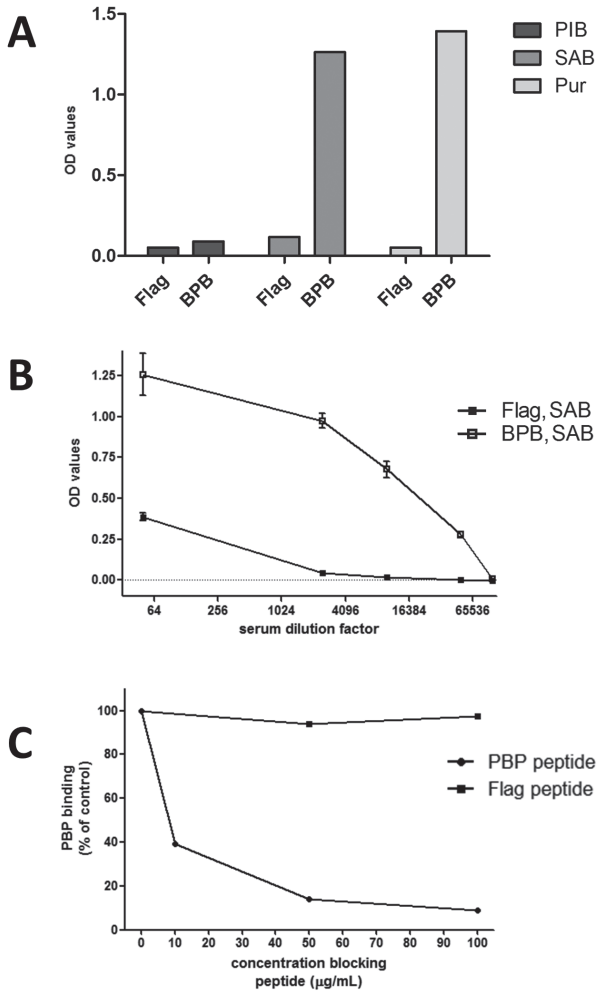

Figure 1. An assay to selectively and sensitively measure anti-PBP antibodies.
(A) Neutravidin-plates were coated with biotin-PBP (PBP) or biotin-Flag (Flag) peptide, and wells were incubated with rabbit pre-immunisation blood serum (PIB), final bleed serum (SAB) or purified antibodies. No positive signal is detected in rabbit serum before immunization with AKEERRY tandem peptide, but specific reactivity against PBP, but not Flag, is detected after two rounds of immunization.
(B) Serial dilutions of rabbit post-immunization serum (SAB) were performed on PBP-peptide or Flag-peptide-coated Neutravidin plates, showing excellent sensitivity of PBP-peptide detection over a-specific binding.
(C) A 1:20,000 dilution of SAB serum was incubated on PBP-peptide-coated Neutravidin plates. Preincubation of sera with increasing concentration of PBP peptide resulted in increasing competition for antibody binding to the plates. In contrast, addition of Flag peptide to the sera did not inhibit antibody binding to PBP coated plates, demonstrating the specificity of the PBP-peptide:PBP-antibody interaction.

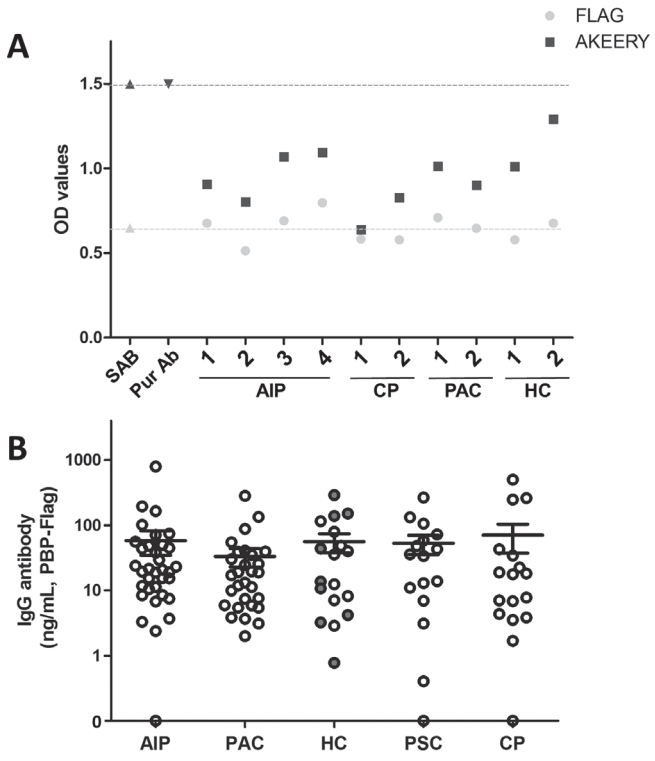

Figure 2. No difference in anti-PBP reactivity between AIP and other patient groups.
(A) Raw ELISA results of a selection of serum samples of individual patients; reactivity against PBP and Flag peptide is shown, indicating patient variability.
(B) ELISA results were compared to standard curves and specific PBP reactivity is shown for all individual patients. Patient group mean±SEM is indicated. *H. Pylori*-positive healthy controls are indicated by grey circles. SAB indicates PBP-immunized rabbit serum; Pur Ab purified antibodies; AIP autoimmune pancreatitis; CP chronic pancreatitis; PAC pancreatic adenocarcinoma; HC healthy control; PSC primary sclerosing cholangitis.

DISCUSSION

In 2009, Frulloni *et al* identified a novel serological marker for AIP, by screening a random peptide library [18]. This peptide showed homology with plasminogen-binding protein (PBP) of *Helicobacter pylori* as well as the pancreatic enzyme UBR2. These findings potentially provided a link between *H.pylori* infection and AIP. They would support the theory of microbial exposure and antigenic mimicry as a pathogenic mechanism in AIP – i.e. antibodies directed against bacterial components which resemble physiological proteins subsequently harm the body in an autoimmune response. *H.pylori* is associated with several autoimmune conditions like primary biliary cirrhosis, Sjögren syndrome, and autoimmune hepatitis, and molecular mimicry has been proposed as underlying basis for these diseases as well. The same relation is now suggested for autoimmune pancreatitis [22-24]. Serum antibodies recognising a synthesized

bacterial peptide (PBP peptide AKEERRY) were shown to have a sensitivity of 94% and specificity of 95% in identifying AIP patients from PAC patients. While promising, these results have not yet been confirmed by others and this test has not found wide spread implementation in the clinic. In the present study, using a sensitive and selective ELISA-based assay, we did not find increased serum antibodies against the PBP peptide AKEERRY in AIP patients.

Implementation of a novel diagnostic tool requires a reproducible assay, which can be performed in multiple labs world-wide with comparable results. While the previous study used Delphia technology to sensitively measure anti-PBP antibodies, this technology is not available in many facilities, including ours. We therefore aimed to set up an assay that could find a wider application. As small peptides are notoriously poor at binding to plastic surfaces, and since binding differences between wells as well as experiments and facilities may hamper reproducibility, we employed biotin-labelled peptide to coat Neutravidin-plates at saturating levels. This method allows quantitative measurement of nanograms of anti-PBP antibodies. While we did find positivity in some serum samples, we did not observe differences between AIP and PAC patients, or *H.pylori* positive and negative healthy donors. Unfortunately, we were not able to cross-test serum samples from the two studies with the two different methods. While it is theoretically possible that we have included mainly *H. Pylori*-negative AIP patients, this seems unlikely, given the known prevalences in our country[25]. Based on these prevalences in the healthy population, at least 46% of AIP patients would be expected to be *H. pylori*-positive, which would still be lower than the 83% reported in by Frulloni et al. However, in the latter study there was not a one-on-one correlation between *H. Pylori* positivity and serum positivity for PBP peptide.

As our results do not point towards a diagnostic role for PBP-antibodies in AIP, the search for other biomarkers continues. Recently, another type of chronic antigen exposure has been described, which would plead for an antigen-driven immune process[26]. High rates of potential chronic occupational antigen exposure to solvents or industrial or metal dusts were found in two independent AIP cohorts who proved to be composed mainly of blue collar workers. The investigators suggest that this exposure may play a role in the initiation and/or maintenance of IgG4-RD in susceptible individuals. These findings could provide an interesting insight into the unknown pathophysiology of AIP or IgG4-related disease, and suggest that while disease epitopes may be diverse (hampering the identification of novel markers for a diagnostic tool), the search for these epitopes should potentially focus on environmental antigens.

In conclusion, we could not confirm detection of serum anti-PBP antibodies as a potential useful diagnostic tool in AIP.

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CHAPTER 6

IGG4-RELATED PROSTATITIS: A CASE-CONTROL STUDY FOCUSING ON CLINICAL AND PATHOLOGIC CHARACTERISTICS

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ABSTRACT

Objective

To evaluate the occurrence and histopathological characteristics of IgG4-related prostatic involvement in patients diagnosed with AIP.

Methods

Nine cases of IgG4-related prostatitis were identified among 117 males in the autoimmune pancreatitis and IgG4-associated cholangitis patient databases in two tertiary hospitals. Clinical information was retrieved and available prostatic tissue samples and 18 prostatitis control samples were evaluated for characteristic IgG4-related disease (IgG4-RD) features: maximum number of IgG4+ cells/HPF; dense lymphoplasmacytic infiltrate; fibrosis, arranged at least focally in a storiform pattern; phlebitis with or without obliteration of the lumen; and increased number of eosinophils.

Results

The aspecific sign of urine retention was commonly present in IgG4-RD patients with prostatic involvement. In these patients with IgG4-related prostatitis, the median number of IgG4+ cells in prostatic tissue was 150 (IQR20-150) per high power field, compared with a median of 3 (IQR1-11) in control patients ($p=0.008$). Dense lymphoplasmacytic infiltrate was observed in the majority (86% in cases and 72% in control patients) of tissue samples independent of the underlying cause of prostatitis. Fibrosis in at least a focally storiform pattern was seen rarely in both groups and (obliterative) phlebitis was absent in all patients. Furthermore, eosinophil numbers were more often elevated in patients with IgG4-RD compared to controls ($p<0.001$). In two cases amelioration of the prostatitis symptoms upon corticosteroid treatment was documented.

Conclusion

Prostatic involvement may not be rare in patients with pancreatic and/or biliary IgG4-RD. Clinicians should consider this disease entity in patients with IgG4-RD and prostatic symptoms.

INTRODUCTION

Prostatitis may be due to infectious and non-infectious causes, of which chronic abacterial prostatitis, or chronic pelvic pain syndrome, is by far the most common[1]. IgG4-RD is a rare systemic disorder that is best known for its manifestations in the pancreas (autoimmune pancreatitis, AIP) and biliary tree (IgG4-associated cholangitis, IAC)[2,3]. However, several other organs may be involved, including the kidney and prostate (Supplementary table 1)[4-7]. Most patients with IgG4-RD have elevated serum levels of IgG4 and increased numbers of IgG4-positive plasma cells in tissue[8]. Disease activity can usually be adequately repressed by corticosteroid therapy[2].

The diagnosis of the disease, which in most affected organs closely mimics malignant disease, can be extremely challenging, both due to the absence of an adequately sensitive and specific test and to limited insight in the clinical phenotype. This certainly holds true for the prostate manifestation of IgG4-RD, first published in 2006, which in many cases may remain undetected. Based on prostate imaging, it is not possible to distinguish IgG4-related prostatitis from other types of prostatitis. As IgG4-RD typically responds well to corticosteroid treatment[9,10], recognition of IgG4-related prostatitis may enable adequate treatment in these patients and could thus avoid unnecessary (surgical) interventions.

An initial case of what appeared to be IgG4-related prostatitis in one of our patients focused our attention to this disease entity. As it is known that IgG4-RD can often simultaneously affect multiple organs in a single patient, we reasoned that we might be able to detect prostate localizations of IgG4-RD in our cohort of patients with pancreatic and/or biliary manifestations of the disease. We thus aimed to investigate in a larger cohort of patients with IgG4-RD the occurrence and histopathological characteristics of IgG4-related prostatitis.

MATERIAL AND METHODS

Patients and controls

Identification of cases with IgG4-RD prostatitis

The index case (case 1) was identified based on non-prostate imaging and remarkable response to therapy. A cross search for patients with prostatitis symptoms mentioned in the patient files was then performed in the AIP and IAC patient registry of two tertiary centers in The Netherlands, containing a total of 117 patients diagnosed with either disease between 1992 to 2012. As controls, prostatic biopsies of patients with prostatitis with or without benign prostatic hyperplasia but without a history of IgG4-RD of any organ were used. The study was approved by the Institutional Review Board of the Erasmus MC University Medical Center.

Histopathological examination

Hematoxylin-eosin-stained prostatic tissue of case and control patients was evaluated for the following characteristic features, as described by Desphande *et al*[8]: (dense) lymphoplasmacytic infiltrate; fibrosis, arranged at least focally in a storiform pattern; phlebitis with or without obliteration of the lumen; and increased number of eosinophils. Routine IgG4 immunostaining was performed using monoclonal IgG4 antibody on formaline-fixed, paraffin-embedded

tissue. All cases were evaluated by 4 investigators blinded to the patient's diagnosis and led by two senior pathologists, of whom one is the national expert on pathology of IgG4-RD and one is specialized in prostate histology. In case of opposing views, consensus was reached by discussion.

Statistical analysis

Comparison of categorical data between cases and controls was performed using the Fisher's exact test. For continuous variables, comparisons were made using the Kruskal-Wallis test.

RESULTS

Case presentation

To illustrate the clinical presentation of IgG4-related prostatic disease we here present one of our patients in more detail.

A 39-year old man was referred to the urological outpatient clinic in 2007 with symptoms of urinary hesitancy. Urine sediment, culture and flowmetry did not reveal any abnormalities and serum PSA was 0.2 ng/mL. Since his symptoms were mild, no specific therapy was instituted.

In 2008, he presented at the outpatient clinic of gastroenterology with upper abdominal pain, loss of appetite and 8 kg weight loss. A diagnosis of AIP was suspected, based on a diffuse pancreatic enlargement with rim enhancement on computed tomography imaging, an elevated serum IgG4 level of 1400 mg/dL (normal<140 mg/dL). Furthermore, his obstructive urinary symptoms had markedly increased in severity and computed tomography (CT) of the abdomen (Figure 1) revealed an enlarged, homogenous prostate with ascites in the small pelvic region.

Treatment with prednisolone 40 mg/day was instituted; the patient reported total disappearance of his urinary problems within 4 days and of his other symptoms within 3 weeks. Abdominal CT after 2 months showed significantly decreased prostate and pancreatic swelling and complete disappearance of ascites in the small pelvic region (Figure 1). The prednisolone dose was gradually tapered and stopped after one year. In the following three years his medical condition remained stable and he was free of urinary or other complaints.

Case series

A total of nine cases of prostatic IgG4-RD were identified, based on histology, non-prostate imaging, or response to therapy. Clinical findings are summarized in Table 1. The patients were males (age 39-74), with 4/9 (44%) patients previously diagnosed with autoimmune pancreatitis or cholangitis as a manifestation of IgG4-RD, presenting with severe symptoms of urine retention. In 5/9 (56%) patients IgG4-related autoimmune pancreatitis or cholangitis was preceded by obstructive urinary symptoms in the foregoing 3 years. Serum PSA was elevated in two patients (22%), serum IgG4 in 7/9 (78%) patients.

Four out of 9 (56%) patients underwent transurethral resection of the prostate (TURP) and one suprapubic prostatectomy (SPP) for release of obstructive symptoms. In two patients (22%) needle biopsies were taken for exclusion of prostate adenocarcinoma. The majority of these 9 patients were retrospectively diagnosed with IgG4-prostatitis, based on histopathological

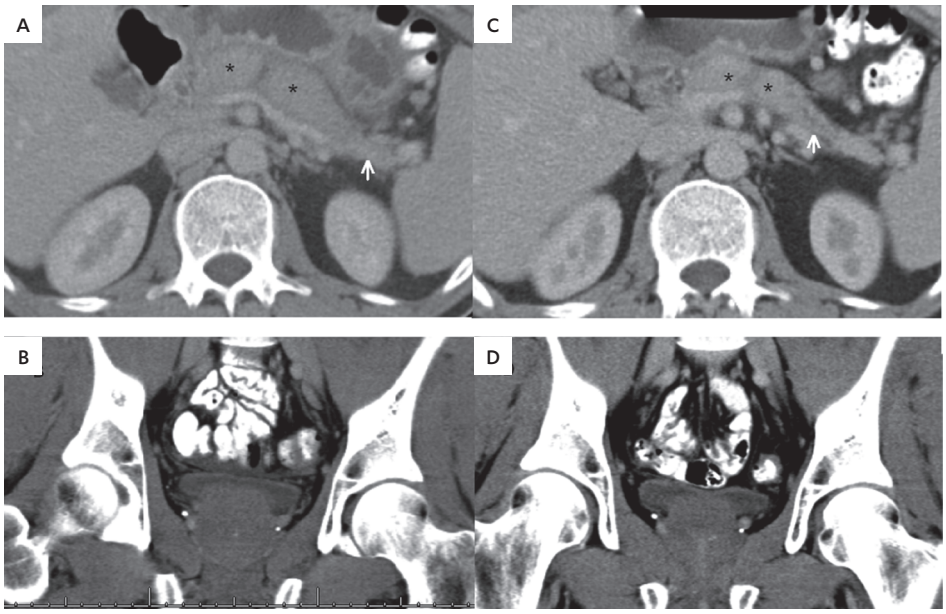

Figure 1. Contrast enhanced abdominal CT in a 39-year old man before and after steroid treatment 2 months later. **(A)** Sausage-shaped pancreatic body (*) with subtle hypodense capsular rim. The pancreatic tail (short white arrow) is relatively atrophic. **(B)** Enlarged prostate (51 x 49 x 49 mm) of homogeneously low density. **(C)** After steroid treatment the pancreatic body (*) has decreased to normal size, although still with loss of normal lobularity. Note irregular and slight pancreatic duct dilatation in atrophic tail (short white arrow). **(D)** Decrease of prostate enlargement (45 x 47 x 44 mm) and return of zonal distinction after steroid treatment.

Table 1. Clinical characteristics of patients with IgG4-related prostatitis

Case	Age (years)	LUTS	Interval between AIP and prostatitis	Serum IgG4 (mg/dL) †	Serum PSA (ng/mL) ‡	Specimen type	Response to prednisolone
1	39	yes	9 mo*	1400	0.2	none	yes
2	67	yes	2 y	1320	2.3	none	yes
3	69	yes	3 y	5	4.2	TURP	not treated
4	64	yes	2 y*	570	7.4	TURP	not treated
5	74	yes	3 y*	613	54	SPP	not treated
6	67	yes	1 mo	1830	0.56	TURP	not treated
7	63	yes	3 mo	90	0.5	NB	not treated
8	74	yes	2 mo*	832	1.23	TURP	not treated
9	72	yes	2.5 y*	733	6.6	NB	not treated

† Normal value, 8-140 mg/dL; ‡ normal value, <2.5 ng/mL in males aged 40-49 y, <3.5 ng/mL if 50-59 y, <4.5 ng/mL if 60-69 y, < 6.5 ng/mL if 70-79;
* Prostatitis before the diagnosis of AIP.
LUTS indicates lower urinary tract symptoms; AIP, autoimmune pancreatitis;
N, normal value; NA, data not available;
TURP, transurethral resection of the prostate; SPP, suprapubic prostatectomy; NB, needle biopsy;

findings. Therefore, no steroid therapy was instituted specifically aimed to reduce prostatic complaints. However, steroid therapy leading to improvement of obstructive symptoms is described in the case presented above. A second patient developed severe urinary tract symptoms, which he himself ascribed to the discontinuation of steroid treatment because the symptoms arose within a few days of steroid cessation. There was no suspicion of prostatic cancer according to the attending urologist based amongst others on digital rectal examination, so there was no need to obtain tissue. After several drugs were instituted without result, the patient requested restart of steroid treatment and within days his lower urinary tract symptoms (LUTS) resolved (Table 1).

Histopathological results

An overview of the results of histopathological examination of prostatic tissue is presented in Table 2. Dense lymphoplasmacytic infiltrate was present in 6/7 (86%) cases and 13/18 (72%) control patients. Fibrosis in a storiform pattern was seen in one patient in both groups and phlebitis in none of the patients. An increased number of eosinophils was seen in 6/7 cases and 0/18 control patients (p<0.001). Significant differences were found in the number of IgG4-positive cells/HPF, with a median of 150 (IQR 20-150) in the cases and 3 (IQR 1-11) in the control patients (p=0.008).

Table 2. Histopathological characteristics of prostatic tissue of cases and controls (total n=25)

	Cases	Control patients	p-Value
Number of patients	7	18	
Tissue obtained by:			0.999[2]
Resection, no (%)	5 (71%)	12 (67%)	
Biopsies, no (%)	2 (29%)	6 (33%)	
IgG4 staining of tissue:			
Number of IgG4+ cells/HPF, median (IQR)	150 (20-150)	3 (1-11)	0.008*[,3]
IgG4-RD histopathological features observed in prostate tissue:			
Dense lymphoplasmacytic infiltration, no (%)	6 (86%)	13 (72%)	0.637[2]
Fibrosis, at least focally in a storiform pattern, no (%)	1 (14%)	1 (6%)	0.490[2]
Phlebitis, with or without obliteration of the lumen, no (%)	0 (0%)	0 (0%)	N.A.
Increased number of eosinophils[1], no (%)	6 (86%)	0 (0%)	<0.001*[,2]

[1] Increased number of eosinophils: > 10/HPF; [2] Fisher's exact test; [3] Mann-Whitney test; * p-Value < 0.05; NA: not applicable

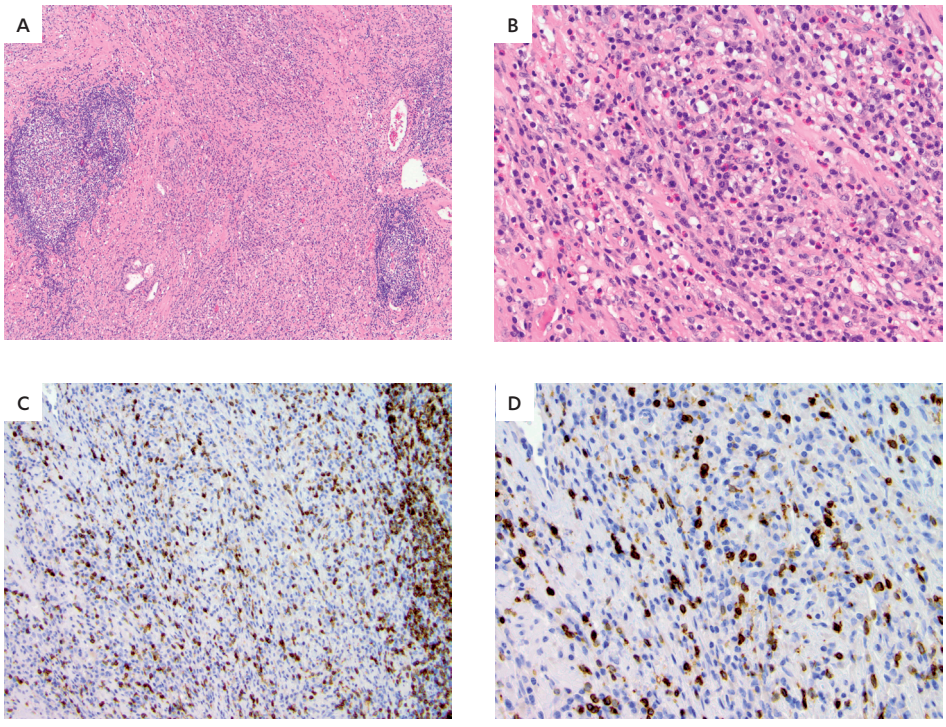

Figure 2. Trans urethral resection of the prostate (TURP) showing IgG4-related prostatitis. 40x and 200x magnification showing patchy lymphoplasmacytic infiltrations with increased number of eosinophils (**A**, **B**). 100x and 200x magnification with immunohistochemical stain for IgG4 shows many (>40/HPF) IgG4-positive cells (**C,D**).

COMMENT

We here describe, to our knowledge, the largest series of cases with prostatic involvement in patients with IgG4-RD. Although the study design did not allow for the identification of any urological symptoms specific for IgG4-related prostatitis, we did find 9 patients with suspected prostatic involvement among a total of 117 patients with IgG4-related pancreaticobiliary disease. We observed marked differences between the prostate tissue of patients with IgG4-RD and control prostatitis patients: tissue infiltration with IgG4-positive plasma/B cells and eosinophils were characteristic histological features in patients with prostatitis associated with IgG4-RD. Our findings show that the prostate represents an organ more often affected by IgG4-RD than previously assumed and these data may help physicians that treat patients with systemic IgG4-RD to recognize prostatic involvement. Furthermore, our case series stresses that urological symptoms may precede other organ manifestations, and detection of IgG4-related prostatitis in these cases could speed up the diagnostic and therapeutic process in these patients.

Little is known on IgG4-related prostatitis. Until now a total of ten patients have been reported in the literature (Supplementary Table 1)[4-7]. Our case series shows that the clinical phenotype, serological and pathological features among these nine patients are comparable to the previous reported cases, strengthening the concept of IgG4-related prostatitis as a clinical entity.

Diagnosing IgG4-RD in all cases relies on the combined approach of excluding other causes for the observed disease pattern and the application of positive criteria for the presence of IgG4-RD, of which the HISORt-criteria[11,12] are most widely applied in the US and Europe. In the pancreas and biliary tree it is well-documented that IgG4-RD lesions may strongly mimic malignant processes and this appears to be the case as well in the prostate[13,14]. Up to now, serum IgG4 is the best single test to diagnose IgG4-RD. However, sensitivity and specificity are limited with elevated serum IgG4 levels present even in malignant disease[15-18]. Clinicians thus need to fully exclude other causes for the disease and furthermore gather pieces of evidence that support the diagnosis of IgG4-RD[19]. A careful history taking or IgG4 immunohistochemistry on historic biopsy specimens may reveal earlier episodes suggestive of IgG4-RD in the same or other organs.

The histological interpretation of tissues affected by IgG4-RD remains challenging. Using the most widely accepted criteria as defined by Deshpande et al.[20] we observed that the prostate lesions in IgG4-RD patients showed elevated numbers of IgG4-positive plasma/B cells and eosinophils per HPF, in line with earlier descriptions in other organs affected by IgG4-RD. The cut-off value of 20 IgG4-positive cells per HPF that is used in the pancreas seems adequate for the prostate. It should be noted that sampling issues regularly are a problem in the histological evaluation of IgG4-RD tissue due to the patchy distribution of the IgG4-positive B/plasmacellular infiltrate. In our study, many resection specimens were available, which may not always be the case in clinical practice.

Storiform fibrosis, once considered a hallmark of IgG4-RD pathology, was not found to be significantly present, as it has earlier been reported to be absent in organs like lymph node[21], lung[22], or lacrimal glands[23]. These differences are in line with our earlier observations in other tissues and with the reported variability across the different organs[12,24,25]. Pathologists should thus be aware of IgG4-RD in the prostate, when conspicuous infiltration of lymphocytes and plasma cells together with eosinophils is observed.

In line with the earlier published cases of IgG4-prostatitis we have documented several patients in which steroid therapy did improve symptoms. As we detected most patients in a retrospective fashion, though, the bulk of patients did not receive immunosuppressants targeted at the urological symptoms, making it impossible to draw conclusions regarding the efficacy of treatment to normalize prostate functioning. Ideally, this should be investigated in a prospective way.

Furthermore, one of the main characteristics of IgG4-related disease is the dramatic response to steroid therapy[2]. It seems unlikely that IgG4-related prostatitis will be an exception in this context. Therefore, in patients with a suspicion of IgG4-related prostatitis and not responding to steroid therapy, this diagnosis should be reconsidered.

This case series could imply that a proportion of patients currently diagnosed with chronic prostatitis or chronic pelvic pain syndrome may in fact suffer from a monosymptomatic disease manifestation of IgG4-related prostatitis. Especially in patients with well-established IgG4-RD presenting with LUTS, prostatic involvement should be considered to avoid ineffective medical or unnecessary surgical treatment. As we have in this study identified patients with prostate involvement in our cohorts of IgG4-RD of the pancreas and/or biliary tree, it would equally be interesting to investigate the prevalence of suspected IgG4-related prostatitis among a large cohort of patients with not otherwise explained prostatitis.

We can only speculate upon the mechanisms that underlie the striking range of organs that have been reported as localizations of IgG4-RD. Chronic antigenic exposure could underlie a IgG4-dominant response[26], potentially directed against a self-antigen, but the IgG4 response could also be reactive to chronic immune activation, as was recently shown in malignant melanoma where IgG4 suppresses the IgG1-mediated chronic antitumor-response[18].

CONCLUSIONS

In conclusion, we here present the largest case series of patients with prostate involvement of IgG4-related systemic disease. Our case series shows that the association is not rare and stresses the need for both urologists and pathologists to consider IgG4-RD as a possible cause for prostatic symptoms as well as for physicians treating patients with other organ manifestations of IgG4-RD to be aware of the possibility that the prostate can also be affected by IgG4-RD.

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Supplementary Table 1. Clinical features of earlier reported patients with IgG4-related prostatitis

Reference	Age (years)	LUTS	AIP	Serum IgG4 (g/L) †	Serum PSA (µg/L) ‡	Procedure	Response to steroids
Yoshimura *et al* [1]	65	yes	yes	3.79	N	TURP	yes
Nishimori *et al* [2]	40	yes	yes	10.30	NA	NB	yes
Nishimori *et al* [2]	49	yes	no	4.73	1.62	TURP	not treated
Uehara *et al* [3]	66	yes	yes	15.50	5.5	RP	not treated
Uehara *et al* [3]	73	yes	yes	14.35	7.2	NB	not treated
Uehara *et al* [3]	71	yes	yes	4.99	1.57	NB	yes
Uehara *et al* [3]	55	yes	yes	14.40	0.38	NB	yes
Uehara *et al* [3]	73	yes	yes	11.60	5.84	NB	not treated
Uehara *et al* [3]	66	yes	yes	13.00	0.1	NB	yes
Hart *et al*[4]	55	yes	yes	10.00	0.67	NB	yes

† Normal value, 0.08-1.40 g/L; ‡ normal value, <2.5 µg/L in males aged 40-49 y, <3.5 µg/L if 50-59 y, <4.5 µg/L if 60-69 y, < 6.5µg/L if 70-79;
LUTS indicates lower urinary tract symptoms; AIP, autoimmune pancreatitis; N, normal value; NA, data not available; TURP, transurethral resection of the prostate; RP, radical prostatectomy; NB, needle biopsy;

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CHAPTER 7

COMPARABLE EFFICACY OF LOW- VERSUS HIGH-DOSE INDUCTION CORTICOSTEROID TREATMENT IN AUTOIMMUNE PANCREATITIS

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ABSTRACT

Objective

The objective of this study was to compare efficacy of high versus low doses of prednisone for induction of remission in autoimmune pancreatitis (AIP).

Methods

This is a retrospective, multicenter study including patients diagnosed with AIP between May 1992 and August 2011. Clinical, laboratory and imaging findings were assessed before treatment and at 1, 3, and 6 months after starting treatment.

Results

A total of 65 patients (57 males; median age, 63 years) were treated with an initial low dose (10-20 mg/d, n = 14), a medium dose (30 mg/d, n = 15) or a high dose (40-60 mg/d, n = 36) of prednisone. There were no significant differences in baseline characteristics between the treatment groups including age, presenting symptoms and laboratory results. During a follow-up period of 6 months, in nearly all patients symptoms (jaundice, weight loss) resolved completely. After 6 months, treatment response with respect to symptomatic, radiological and laboratory improvement was comparable for the different dosage groups.

Conclusions

Response to therapy was comparable for AIP patients treated with doses of prednisone in the range of 10 to 60 mg/d. A prospective trial should be conducted to confirm efficacy of lower-dose prednisone treatment.

INTRODUCTION

Autoimmune pancreatitis (AIP) is a distinct type of chronic pancreatitis, predominantly affecting males in their fifth and sixth decade. Frequently AIP represents the pancreatic manifestation of immunoglobulin G4 (IgG4)-related disease, a systemic disorder that may involve not only the pancreas but also almost any other organ. Patients frequently present with obstructive jaundice, weight loss, steatorrhea, and diabetes mellitus. Characteristic radiological features include diffuse enlargement of the pancreas and irregular narrowing of the main pancreatic duct.

Laboratory tests often reveal elevated serum levels of IgG and/or IgG4[1]. Histologically, AIP is frequently associated with a lymphoplasmacytic infiltration rich of IgG4-positive plasma cells and fibrosis of the pancreatic parenchyma[2].

Unlike other types of pancreatitis, AIP responds dramatically to steroid therapy[3-7]. The usually recommended dosage of prednisone (or equivalent dosage of prednisolone) for remission induction is 0.6 mg/kg per day, resulting in daily starting doses of 30 to 40 mg[8-10]. This recommended dosage is largely based on empirical data but lacks a solid scientific basis. Corticosteroid treatment, in particular when high doses are used, is potentially associated with significant side effects[11-14]. These negative treatment effects may even be more important in patients presenting with AIP because this is a population characterized by relatively advanced age, (de novo) diabetes mellitus, and obstructive jaundice.

Furthermore, the rationale for high-dose treatment could be questioned considering the well-established sensitivity of AIP to corticosteroids. We therefore investigated the efficacy of treatment in AIP patients using low (≤20 mg), medium (30 mg), and high (≥40 mg) daily doses of prednisone therapy for induction of remission.

METHODS

Patients and treatment

A retrospective, multicenter study was conducted among patients diagnosed with AIP between May 1992 and March 2012 in 4 centers in the Netherlands. Data were retrieved from electronic medical record systems and by reviewing paper hospital charts.

Patients were included if they fulfilled the International Consensus Diagnostic Criteria, Asian or HISORt (Histology, Imaging, Serology, Other organ involvement and Response to therapy) diagnostic criteria for AIP, or if diagnosis could be based on post-surgery histology, a combination of unexplained pancreatic disease, biliary disease/extrapancreatic manifestations and either response to steroids or IgG4-positive serology, and had been treated with prednisone therapy for induction of remission[7, 15, 16].

All patients were treated with oral prednisone, not according to a particular multicentre protocol but at the discretion of the treating physician. In patients treated with 10 or 15 mg per day, this dose was maintained for at least 6 months. In 3 other centers, patients were generally treated with 30 to 40 mg prednisone per day during 2 to 4 weeks after which the dose was tapered, usually with 5 mg per 1 to 2 weeks.

Patients were excluded when (1) essential data with respect to the dose and duration of treatment and the evolution of symptoms, radiological abnormalities, and laboratory findings

were not available; (2) they had previously been treated with corticosteroids for the same condition; (3) concurrent initial treatment with azathioprine or other immunomodulating agents was instituted during the first three months because this was considered to hamper interpretation of the results; (4) when biliary stents were still in place 6 months following the start of treatment, to exclude confounding by biliary drainage on clinical and biochemical response (Figure 1).

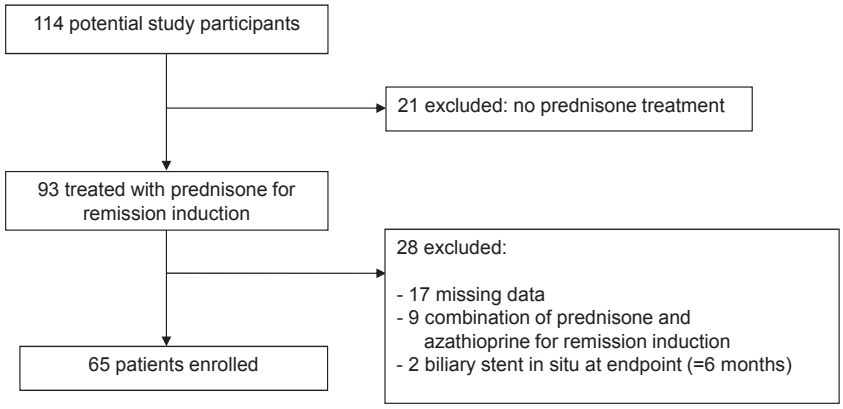

Figure 1. Study enrollment and exclusion.

Data regarding induction of remission by steroid treatment were collected immediately before starting treatment (maximal allowed period 4 weeks) and subsequently after 1, 3, and 6 months.

In addition, data on concurrent biliary drainage, timing of stent removal, and other immunosuppressive drugs, which were initiated during the follow-up period, were analyzed.

Symptomatic response was defined as the disappearance of the initial clinical symptoms. Radiological response was defined as marked improvement or resolution of the pancreatic and/or extrapancreatic manifestations on imaging studies, particularly pancreatic swelling and pancreatic and biliary duct strictures. Relapse was defined as recurrence of disease after discontinuation of steroid therapy.

Laboratory evaluation

The following laboratory parameters were analyzed: serum levels of IgG4, IgG, total bilirubin, alkaline phosphatase (ALP), aspartate aminotransferase (ASAT), and alanine aminotransferase (ALAT).

Imaging

Initially patients were examined by contrast-enhanced computed tomography (CT), magnetic resonance imaging (MRI), and/or endoscopic retrograde cholangiopancreatography (ERCP). Morphological changes after steroid therapy were studied with CT and MRI or ERCP.

Complete remission

We used the following criteria to evaluate complete remission in the different dosage groups: disappearance of clinical symptoms and resolution of pancreatic abnormalities on imaging studies.

Statistical analysis

Data were analyzed in several ways. First, we analyzed data with prednisone as a continuous variable. We also analyzed data with patients categorized into 2 prednisone dosage groups (≤20 and >20 mg/d). Finally, data were analyzed with patients categorized into 3 prednisone dosage groups (low dose: 10-20 mg/d, medium dose: 30 mg/d and high dose: 40-60 mg/d). Results are presented with patients categorized into 3 dosage groups (low, medium, and high).

Statistical analysis was performed using Fisher exact test and Kruskall-Wallis test using SPSS 17.0 to compare baseline characteristics between patients treated with different doses of prednisone.

Differences in symptomatic and radiologic response between groups treated with different initial doses of prednisone were compared using Fisher exact test. Differences in biochemical response between the treatment groups were compared in a repeated measurement model with a random intercept and random decline from baseline to month 1 and a random linear decline from month 1 and onwards to month 6. This broken stick model was used to describe the observed changes in the 2 described periods. The random intercept allows for adjustment of the individual baseline biochemical values.

To correct for multiple testing, $P < 0.01$ was considered statistically significant.

Medical ethical concerns

The study was approved by the Institutional Review Board of the Erasmus University Medical Center, Rotterdam, the Netherlands.

RESULTS

Patients

A total of 65 patients with AIP (57 men and 8 women with a median age of 63 years) were included (Figure 1). A recent onset of diabetes mellitus (<1 year) was seen in 24 (38%) of 65 patients. Extrapancreatic manifestations were observed in 48 (74%) of 65 patients, including IgG4-related sclerosing cholangitis in 38 (59%) of 65 cases. Table 1 provides a further overview of demographic data and clinical symptoms.

No patients were lost to follow-up during the six months' study period. Five patients were treated with an initial dose of prednisone of 10 mg/d, 2 patients with 15 mg/d, 7 patients with 20 mg/d, 15 patients with 30 mg/d, 34 patients with 40 mg/d, and 2 patients with 60 mg/d. The mean prednisone induction dosage in the low dose group (10-20 mg) was 0.22 mg/kg per day, in the medium dose group (30 mg) was 0.41 mg/kg per day and in the high dose group (40-60 mg) was 0.55 mg/kg per day.

There were no significant differences in baseline characteristics including gender, age, presenting symptoms, laboratory, and imaging results between the treatment groups (Table 1).

Table 1. Baseline patient characteristics

	AIP patients (n = 65)	Low Dose (n = 14)	Medium Dose (n = 15)	High Dose (n = 36)	Dose Categorized: Low-Medium-High (*P*)
Male, n (%)	57 (88)	13 (93)	12 (80)	32 (89)	0.60*
Age at onset, median (IQR), y	63 (53-71)	67 (62-74)	59 (49-66)	62 (44-72)	0.02†
Weight, median (IQR), kg	74 (70-83)	72 (58-84)	74 (67-79)	74 (70-87)	0.42†
Initial symptoms, n (%)					
Jaundice	47 (73)	11 (79)	11 (73)	25 (71)	0.93*
Weight loss	55 (87)	13 (93)	12 (80)	30 (88)	0.68*
Recent-onset diabetes mellitus	24 (38)	6 (43)	5 (33)	13 (37)	0.89*
Steatorrhea	40 (69)	12 (86)	12 (80)	16 (55)	0.10*
Abdominal discomfort	34 (53)	6 (43)	7 (47)	21 (60)	0.50*
Laboratory tests, median (IQR)					
IgG4, g/L (n ≤ 1.40)	5.4 (1.7-11.0)	8.6 (5.4-20.6)	3.3 (0.6-8.0)	5.0 (1.0-9.6)	0.02†
Elevated IgG4, n (%)	41 (77)	11 (100)	7 (70)	23 (72)	0.11*
IgG, g/L (n ≤ 16.0)	16.1 (11.5-20.8)	20.0 (14.4-28.1)	21 (8.7-28.6)	14.5 (11.3-17.4)	0.04†
Total bilirubin, µmol/L (n ≤ 16)	49 (14-151)	42 (17-150)	79 (18-182)	49 (13-138)	0.78†
ALP, U/L (n ≤ 114)	425 (235-618)	564 (309-768)	468 (333-794)	379 (203-590)	0.20†
ASAT, U/L (n ≤ 34)	126 (55-186)	139 (77-168)	146 (72-176)	93 (38-223)	0.63†
ALAT, U/L (n ≤ 44)	129 (67-306)	130 (65-213)	137 (112-461)	127 (49-337)	0.53†
Radiology, n (%)					
Pancreatic enlargement					0.68*
Diffuse	34 (58)	6 (46)	6 (50)	22 (65)	
Focal	16 (27)	4 (31)	4 (33)	8 (24)	
Diffuse narrowing pancreatic duct	22 (54)	4 (80)	4 (44)	14 (52)	0.55*
Extrapancreatic lesions, n (%)	48 (74)	12 (86)	11 (73)	25 (69)	0.55*
IgG4-related sclerosing cholangitis, n (%)	38 (58)	9 (64)	10 (67)	19 (54)	0.54*

* Exact test
† Kruskal-Wallis test

In general, the initial dose was administered for 2 to 4 weeks and gradually tapered by 5 mg every 2 weeks. In patients treated with 10 to 20 mg/d, the initial dose was maintained for a longer period. During the follow-up period, the dose of prednisone was not raised in the low-dose group.

At the time when prednisone was started, 28 (43%) of 65 patients were treated for distal biliary obstruction by endoscopic insertion of plastic endoprotheses: 6 (43%) of 14 patients in the

low-dose group, 5 (33%) of 15 in the medium-dose group and 17 (47%) of 36 in the high-dose group. These stents were removed after a median of 10 weeks (interquartile range [IQR], 5-15) weeks.

During the follow-up period of 6 months, azathioprine was added to steroid therapy in 12 (18%) of 65 patients: 6 of 14, 1 of 15, and 5 of 29 in the low-, medium- and high-dose group, respectively. The shortest interval between initiation of steroid treatment and introduction of azathioprine was 4 months. Seven (58%) of the 12 patients who were treated with azathioprine took this drug during at least 2 months. The number of patients treated with azathioprine during the 6 months follow-up period did not differ between the treatment groups ($P = 0.038$).

Clinical response

During a clinical follow-up period of 6 months, 59 (92%) of 65 patients achieved complete clinical response, whereas in 5 (8%), the response was partial. The regression of clinical symptoms after 6 months of treatment was not associated with the dosage of prednisone ($P = 0.999$) (Figure 2).

During the 6-month follow-up period, all patients in the low-dose group continued prednisone treatment in contrast to 12 of 15 and 20 of 36 in the medium- and high-dose group, respectively ($P = 0.003$). No relapses were observed in those patients in whom prednisone was discontinued.

We also analyzed data with prednisone dosage as a continuous variable and categorized into 2 prednisone dosage groups (≤20 and >20 mg/d). No dose-response correlation or significant differences were found.

Biochemical response

Before steroid therapy was started, elevated levels of serum IgG (>18 g/L) and IgG4 (>1.4 g/L) were observed in 42% and 77%, respectively. After start of treatment, IgG4 showed a rapid

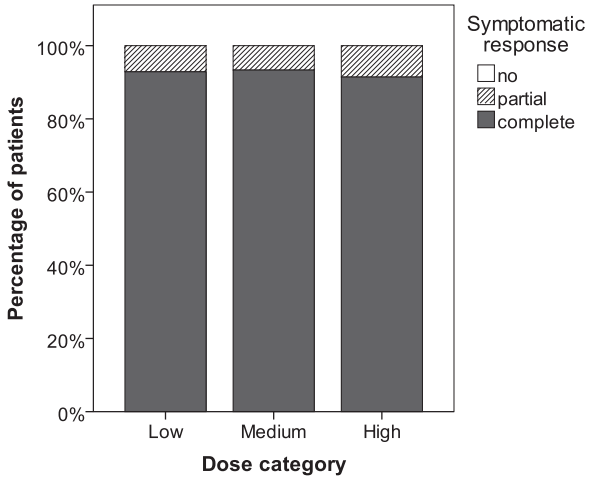

Figure 2. Symptomatic response after 6 months of prednisone therapy for induction of remission. Results were categorized in 3 treatment groups, low dose (10-20 mg/d), medium dose (30 mg/d) and high dose (40-60 mg/d) prednisone.

decline in the majority of patients, but levels remained elevated in 76% (Figure 3A). IgG normalized in all patients (Figure 3B). At baseline, the majority of patients had abnormal

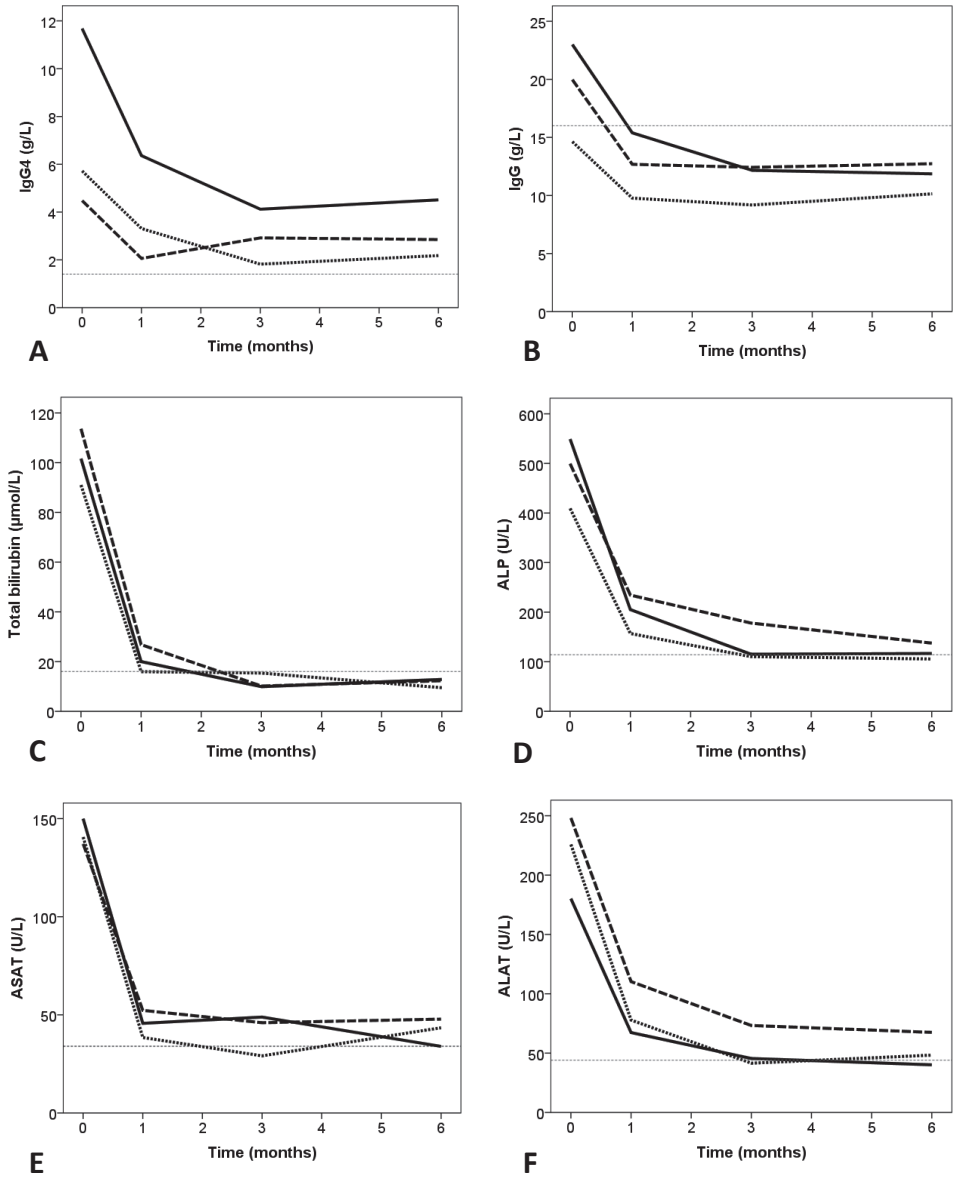

Figure 3. Biochemical mean response during 6 months of prednisone therapy for induction of remission: IgG4 (**A**), IgG total (**B**), Total Bilirubin (**C**), Alkaline Phosphatase (**D**), ASAT (**E**), ALAT (**F**). — low dose (10-20 mg/d), -- medium dose (30 mg/d) and ···· high dose (40-60 mg/d) prednisone. Cutoff levels are indicated by the horizontal lines.

serum liver tests. In conjunction with clinical improvement, in all patients, rapid declines in total bilirubin, ALP, ASAT, and ALAT were observed, which persisted after stent removal (Figure 3C-F). After 6 months, we found that bilirubin completely normalized in 85% of the patients, ALP in 57%, ASAT in 61%, and ALAT in 67%. No significant differences were detected between the 3 treatment groups.

Treatment response as assessed by biochemical parameters was not associated with doses of prednisone (Table 2). Although the quantitative decrease in IgG and IgG4 levels in the low-dose group was more pronounced, no significant differences were observed at 6 months ($P = 0.131$, $P = 0.234$).

Data regarding biochemical response were also analyzed with prednisone as a continuous variable and categorized into 2 groups (≤20 and >20 mg/d). However, these analyses revealed no dose-response correlation or significant differences.

Radiological response

Before treatment, diffuse pancreatic enlargement was observed in 37 (59%) of 63 patients and focal pancreatic enlargement in 18 (29%) of 63 patients. In 22 (71%) of 31 patients, ERCP or MRI showed diffuse narrowing of the main pancreatic duct (MPD), whereas in 3 (10%) of 31 patients, segmental narrowing of the MDP was observed.

After 6 months of steroids, all patients showed partial (19 patients, 42%) or complete (26 patients, 58%) resolution of pancreatic or biliary abnormalities on imaging studies (Figure 4). Radiological response was analyzed with prednisone dosage as a continuous variable and

Table 2. Response to treatment during 6 months of treatment.

	Low	Medium	High	
Biochemical Response	**0-1 mo (95% CI)** **1-6 mo (95% CI)**	**0-1 mo (95% CI)** **1-6 mo (95% CI)**	**0-1 mo (95% CI)** **1-6 mo (95% CI)**	***P***
IgG4, g/L	-4.28 (-7.34 to -1.22) -0.58 (-0.90 to -0.25)	-3.30 (-6.87 to 0.27) 0.22 (-0.30 to 0.73)	-3.49 (-5.44 to -1.53) -0.05 (-0.35 to 0.25)	0.85 0.03
IgG, g/L	-8.15 (-11.29 to -5.00) -0.74 (-1.14 to -0.35)	-7.17 (-11.97 to -3.36) 0.03 (-0.43 to 0.49)	-5.48 (-7.68 to -3.28) 0.13 (-0.29 to 0.54)	0.34 0.01
Total bilirubin, µmol/L	-84.8 (141.5 to -28.2) -1.21 (-3.19 to 0.77)	-91.0(-147.6 to -34.4) -2.10 (-4.20 to 0.01)	-73.1 (-109.0 to -37.2) -1.60 (-3.07 to -0.14)	0.84 0.82
ALP, U/L	-363 (-491 to -234) -16.5 (-30.3 to -2.58)	-294 (-422 to -166) -15.4 (-30.8 to -0.02)	-263 (-345 to -181) -11.4 (-22.0 to -0.81)	0.42 0.82
ASAT, U/L	-101.6 (-167.8 to -35.4) -2.49 (-7.52 to 2.54)	-87.4 (-149.6 to -25.2) -0.57 (-5.92 to 4.77)	-106.7 (-146.8 to -66.6) 0.85 (-3.13 to 4.83)	0.87 0.57
ALAT, U/L	-117.1 (-235.9 to 1.73) -5.16 (-14.09 to 3.76)	-160.0 (-279.5 to -40.3) -5.71 (-15.57 to 4.15)	-158.2 (-234.1 to -82.4) -6.18 (-13.09 to 0.72)	0.82 0.98

Estimated mean decline the first month and from month 1 to month 6 by dosage.
Decline adjusted for baseline biochemical values in a repeated measurement model with a random intercept and random decline from baseline to month 1 and a random linear decline from month 1 and onwards to month 6 [broken stick model y= a + b * t + c (t – 1), where t = months y = lab value].

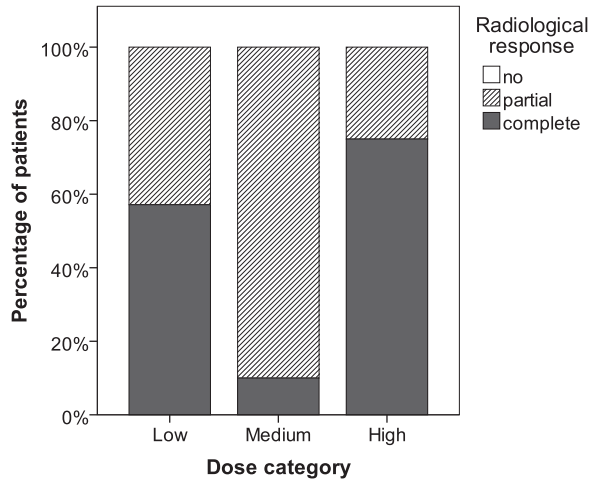

Figure 4. Radiological response after 6 months of prednisone therapy for induction of remission. Results were categorized into 3 treatment groups: low dose (10-20 mg/d), medium dose (30 mg/d), and high dose (40-60 mg/d) prednisone.

categorized into 2 groups (≤20 and >20 mg/d) as well. These analyses did not result in a dose-response correlation or significant differences.

Complete remission

Furthermore, we analyzed the capability for the different dosage groups to achieve complete remission. The rate of complete remission was 71% (10/14) in the low-dose group, 40% (6/15) in the medium-dose group, and 72% (26/36) in the high-dose group.

DISCUSSION

This study shows comparable therapeutic efficacy of low-dose (10-20 mg/d) prednisone as compared to high-dose (40-60 mg/d) prednisone for induction of remission in AIP.

The therapeutic efficacy of corticosteroid in AIP has been well documented[3-7], but we are not aware of previous studies specifically addressing different corticosteroid remission induction regimens. In a number of studies not primarily aimed to evaluate dose-response relationships, comparable outcomes were reported for patients treated with medium to high doses of prednisone, ranging from 25 to 50 mg/d[9, 17-19]. Reports on corticosteroid induction doses lower than 15 mg/d are scarce. One case report described successful treatment with prednisolone 5 mg/d[20].

Although worsening of glycaemic control is a known side effect in the elderly diabetic AIP patient, steroid therapy has been reported to improve endocrine pancreatic function in approximately half of the patients. Yet this beneficial effect is counterbalanced by newly developed diabetes or worsening of diabetic control in a substantial subset of patients[6, 18, 21, 22].

High-dose steroid therapy, especially during an extended period (>1 week), poses a substantial risk for significant side effects[11-14, 23]. Frequently observed important side-effects in elderly populations are inducing, or worsening of pre-existing, diabetes mellitus. Other possible risks include weight gain, increased bone loss, opportunistic infections, and psychological disturbances.

Diabetes mellitus or worsening of glycaemic control is frequent in individuals presenting with AIP[4, 19, 21, 24]. In a cohort of 114 Dutch patients, 35% of patients had recent-onset diabetes at presentation (unpublished data). Corticosteroids, in particular high doses, obviously have the potential to further impair glucose tolerance and glycaemic control. In series of elderly patients with rheumatoid arthritis and chronic kidney disease, 9 to 40% developed diabetes mellitus upon treatment with steroids. Older age and obesity were identified as independent risk factors[12, 13]. In addition, Gurwitz et al[14] demonstrated with prednisone therapy a dose-related risk of developing hyperglycaemia requiring therapy with oral glucocorticoid use. The odds ratio for starting an oral hypoglycaemic agent or insulin ranged from 1.77 for patients treated with a hydrocortisone equivalent dose of 1 to 39 mg/d, to 3.02 for 40 to 70 mg/d and to 5.82 for 80 to 119 mg/d.

Furthermore, high-dose steroids result in a greater risk of complicated glucocorticoid withdrawal and require longer periods of drug tapering. Any patient treated with at least 20 mg/d prednisone for more than 5 days is at risk of hypothalamic-pituitary-adrenal suppression[25].

Our study has a number of limitations. This study had a retrospective, uncontrolled nature and had a limited number of patients treated with low initial doses of prednisone. Importantly, individual patient characteristics or disease manifestations might have influenced the treating physician to choose a particular corticosteroid dose. Nevertheless, as shown in Table 1, there were no significant differences in these baseline characteristics between the treatment groups. It is important to stress that patients included in this series were not treated according to a particular protocol but at the discretion of the treating physician. Most patients in the low-dose group were recently treated by a single physician who believed, based on preliminary observations, that low-dose prednisone could be as effective as higher doses in the initial treatment of the disease. In another center, however, the standard regimen was 30 or 40 mg prednisone per day throughout the study period. Because of the retrospective design of this study, we were not able to retrieve sufficient and/or reliable data for assessing potential adverse treatment effects, for example, on glucose tolerance, body weight, and blood pressure. Concurrent biliary drainage and azathioprine therapy are of concern in interpreting the results of this study.

Pancreaticobiliary imaging after 2 weeks, using CT and/or ERCP, has been recommended to evaluate the response to corticosteroid treatment, in particular when this information is considered part of the diagnostic process[26]. Because AIP often responds well to steroids, biliary stents are often removed at an early stage. Nevertheless, in clinical practice, the timing of stent removal varies substantially, as illustrated by the markedly variable period of stenting in our study. This implies that the observed response to treatment in the first 3 months was due to the combination of steroid treatment and biliary drainage.

The percentage of patients treated with stents and the time to stent removal did not differ between the groups, and all patients were free of biliary stents after 6 months. Therefore it seems unlikely that our main conclusions are invalidated by concurrent endoscopic treatment. Steroid therapy is frequently combined with other types of immunosuppressive drugs. Following relapse or unsuccessful tapering of prednisone, azathioprine is often used in combination with steroids to maintain remission or as a corticoid-sparing immunosuppressant. We excluded patients initially treated with a combination of immunosuppressive drugs. Patients were not excluded when azathioprine treatment was introduced subsequently. Azathioprine was used in a minority of patients and usually after 3 to 4 months of prednisone treatment. Nonetheless, since the effect of azathioprine is assumed to start after 2 to 3 months, it cannot be excluded that azathioprine influenced the observed 6-month treatment response.

The number of patients treated with azathioprine during the follow-up period did not differ between the treatment groups (P = 0.038). Further studies are needed to study the effect of azathioprine on long-term outcome.

In conclusion, in this retrospective series, response to therapy was comparable for AIP patients treated with doses of prednisone in the range of 10 to 60 mg/d. These preliminary data suggest that low-dose (<20 mg/d) prednisone may be effective for induction of remission of AIP, with possibly avoiding the risks of high dose steroid treatment. However, these results await confirmation, ideally by controlled trials comparing the efficacy and tolerance of low- and high-dose induction corticosteroid therapy, before this can be generally recommended.

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CHAPTER 8

THE LONG-TERM IMPACT OF AUTOIMMUNE PANCREATITIS ON PANCREATIC FUNCTION, QUALITY OF LIFE, AND LIFE EXPECTANCY

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ABSTRACT

Objective

To evaluate the long-term outcome of autoimmune pancreatitis (AIP).

Methods

Patients with at least 2 years of follow-up were included. Information was collected regarding disease characteristics, treatment outcome, diagnosed malignancies, and mortality. In addition, pancreatic function and quality of life (QoL) were assessed prospectively.

Results

Hundred-seven patients were included (87% male, 90% type 1), with a median follow-up of 74 months (IQR 49-108). One third was operated for suspected pancreatic cancer (32%). Most patients were (successfully) treated with steroids (83%), but relapses were common (52%), for which no risk factors could be identified. Pancreatic carcinoma was not observed.

Prospective data were obtained from 64%, as 17% had died, 7% were lost to follow-up, and 13% refused to participate. After a median of 75 months (IQR 50-106), 46% still used active treatment. Exocrine and endocrine insufficiency were highly prevalent (82 and 57%, respectively). QoL and survival were not impaired, as compared to a reference population.

Conclusions

Despite an excellent initial treatment response, relapses are common, even in type 2, and almost half of the patients require maintenance therapy. Pancreatic insufficiency is highly prevalent, which calls for active screening. Pancreatic cancer was not observed and QoL and survival is not impaired.

INTRODUCTION

Autoimmune pancreatitis (AIP) is a unique form of pancreatitis with a dramatic response to steroids. Two subtypes are distinguished.[1,2] Type 1 is the classical form, histologically described as 'lymphoplasmacytic sclerosing pancreatitis' (LPSP). It is part of a systemic IgG4-related disease (IgG4-RD), which can involve multiple organs (i.e. biliary tract, kidneys, and salivary glands) and relapses frequently.[3,4] Type 2, or 'idiopathic duct-centric pancreatitis' (IDCP), is histologically characterized by granulocyte epithelial lesions, with few or no IgG4-positive cells.[5] This type is more rare, pancreas-specific, not associated with elevated serum IgG4, and relapses seem to be less common. AIP is increasingly being recognized and several diagnostic criteria have been proposed, including the HISORt criteria (**H**istology, **I**maging, **S**erology, **O**ther organ involvement, and **R**esponse to steroids) and the **I**nternational **C**onsensus **D**iagnostic **C**riteria (ICDC).[1,2]

Little is known about the long-term consequences of AIP. Establishing the diagnosis can be difficult and patients are often wrongly suspected of pancreatic cancer. This may result in invasive (diagnostic) procedures and even major surgery. The impact of such events on quality of life was never investigated. Furthermore, a delayed diagnosis may lead to pancreatic function loss, because prolonged inflammation causes fibrosis and pancreatic acinar and islet cell loss. Another late complication may be the development of cancer. Other forms of pancreatitis harbour an increased risk for pancreatic cancer, and the same association has been reported for AIP.[6,7]

With this study we set out to investigate the long-term outcome of patients with type 1 and type 2 AIP, in terms of treatment response, pancreatic function, quality of life, risk of pancreatic cancer, and mortality.

MATERIAL AND METHODS

Inclusion criteria

This study was approved by the Institutional Review Board of the Erasmus University Medical Center. Patients registered in our prospectively maintained AIP database for at least 2 years, were enrolled.[8] Type 1 AIP patients had to fulfil either the HISORt criteria or the ICDC, based on their complementarity, as we described previously.[1,2,8] Patients with type 2 AIP were diagnosed as proposed by Maire et al, who added criteria for 'probable type 2 AIP', to allow a diagnosis in the absence of histology.[9,10] Definitive type 2 AIP was defined as histologically confirmed idiopathic duct-centric pancreatitis. The diagnosis of probable type 2 AIP was based on a combination of unexplained pancreatic disease, suggestive imaging (a diffusely enlarged pancreas, irregular pancreatic duct), normal serum IgG4 levels, and a positive response to steroids.

Treatment

There was no formal treatment protocol. The induction regimen most frequently used, consisted of prednisolone 30-40 mg/day for 2-4 weeks, after which steroids were tapered off in 2-3 months. Every 3-6 weeks patients were clinically and biochemically evaluated. Imaging studies were performed to document response to therapy and whenever clinically indicated.

For relapses, steroids were restarted, often in combination with azathioprine. Patients who were unable to taper steroids usually received azathioprine as well.

Data collection

Information regarding patient and disease characteristics, treatment, response, and relapses were retrieved from the database. In addition, the incidence of pancreatic and other malignancies were evaluated. Malignancies, diagnosed within one year of the AIP diagnosis were excluded, to preclude any paraneoplastic phenomenon or misdiagnosis. If data were incomplete, we prospectively collected any missing information by reviewing medical records or contacting treating physicians and/or patients by telephone.

Subsequently, all living patients were invited to participate in the prospective part of the study. If informed consent was obtained, pancreatic function and quality of life were assessed between December 2012 and May 2013. To evaluate the exocrine function, a fecal elastase test was performed (enzyme-linked immunosorbent assay, ScheBo-Tech, Wettenberg, Germany). The endocrine function was assessed by measuring fasting serum glucose levels and glycated hemoglobin levels, and by collecting data on medication use. The quality of life was assessed using the European Organization for Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire C30 (QLQ-C30), and the Short Form-36 (SF-36).[11-13]

Definitions

The assay used to measure serum IgG4 varied among the different hospitals, but the upper limit of normal was uniformly set on 140 mg/dl. IgG4-associated cholangitis (IAC) was defined as biliary involvement, either intrahepatic or proximal to the pancreatic head. Distal biliary narrowing was excluded, as it is impossible to distinguish cholangitis from ductal compression at this site.

Treatment response was defined as the disappearance of initial symptoms, in addition to a marked improvement or resolution of pancreatic and/or extrapancreatic manifestations on imaging studies. A relapse was defined as the recurrence of symptoms, together with a reappearance of pancreatic and/or extrapancreatic imaging abnormalities.[10]

Exocrine insufficiency was defined as an elastase-1 value below 0.200 µg/gr feces. Endocrine insufficiency was considered to be present when the fasting glucose level was above 6.9 mmol/l, the HbA1c level was more than 46 mmol/mol (6.4%), or if patients used oral antiglycemic agents or insulin.[14] Quality of life, the occurrence of malignancies, and mortality were compared to an age- and sex-matched Dutch reference population.

Statistical analysis

Outcome measures are presented as medians with an interquartile range (IQR). Patients with type 1 and 2 AIP were compared with the Mann-Whitney U Test for continuous data and the Fisher's Exact Test for categorical data. Survival, cancer free survival, and relapse free survival were calculated using life tables (Kaplan-Meier method). Cox proportional hazards regression was used for univariate analysis of predictors of disease relapse.

Exact logistic regression analysis was performed to determine risk factors for endo- and exocrine insufficiency, adjusted for length of follow-up. QoL scores were compared to a

reference population with a One-sample *t*-test. A p-value <0.05 was considered statistically significant. To evaluate the cancer risk in AIP patients, we compared the observed cancer frequency to the expected frequency, based on the Dutch population-based cancer incidence rates from 1989-2011 (http://www.iknl.nl). The life expectancy of the general Dutch population, matched for age and sex, was obtained from the online database of the Dutch Central Bureau of Statistics (www.cbs.nl).

RESULTS

Baseline characteristics

A total of 107 patients were included (93 male; median age 71 years (IQR 61-78); Figure 1), with a median follow-up of 74 months (IQR 49-108). Patient characteristics are shown in Table 1. Patients had been diagnosed between May 1992 and August 2011. The median diagnostic delay was 5 months (IQR: 2-19). Presenting symptoms were weight loss in 92 patients (89%), obstructive jaundice in 82 (78%) and abdominal discomfort in 61 (57%). At diagnosis, 72 patients (75%) had symptoms of steatorrhea and 38 patients (36%) were treated for diabetes mellitus.

Imaging studies revealed a pancreatic mass in 21 patients (20%) and diffuse pancreatic enlargement in 62 (59%). In 54 patients a pancreatogram was obtained, which showed irregular narrowing of the pancreatic duct in 46 (85%). Other organs were involved in 72 patients (67%), most frequently IgG4-associated cholangitis of the extrapancreatic, usually intrahepatic, biliary tree (58%). In 24 patients, more than one extra-pancreatic manifestation was observed (33%).

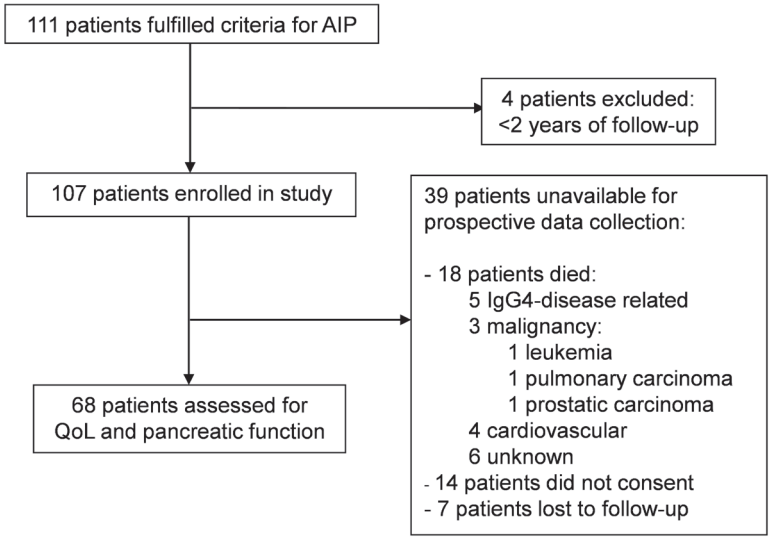

Figure 1. Study enrollment

Table 1. Patient characteristics of entire cohort

Demographics	AIP patients (n=107)	Type 1 AIP (n=96, 90%)	Type 2 AIP (n=11, 10%)	p-value
Age in years - median (IQR)[¥]	71 (61-78)	72 (65-78)	48 (32-61)	<0.001[2,*]
Male sex - no. (%)	93 (87)	86 (90)	7 (64)	0.036[1,*]
Elevated serum IgG4 – no. (%)[¶]	81 (84)	81 (84)	0 (0)	<0.001[1,*]
Other organ involvement, no. (%)	73 (68)	67 (70)	6 (55)	0.321[1]
Inflammatory bowel disease - no. (%)	4 (4)	0 (0)	4 (36)	<0.001[1,*]
Initial treatment:				
Surgery for suspected malignancy - no. (%)	34 (32)	31 (32)	3 (27)	0.999[1]
Pancreatic resection	18 (17)			-
- Diagnostic laparotomy/laparoscopy	14 (13)			-
- Combined gastric and biliary bypass	2 (2)			-
Steroid therapy - no. (%)	89 (83)	81 (84)	8 (73)	-
Relapse	55 (52)	52 (55)	3 (27)	0.114[1]
Azathioprine addition	44 (42)			-
Follow-up in months – median (IQR)	74 (49-108)	75 (50-114)	52 (50-106)	0.261[2]
Death - no. (%)	18 (17)	16 (17)	2 (18)	0.999[1]

[¥] Age at the time of the present study; [¶] At presentation; * p < 0.01; [1]Fisher's Exact Test; [2]Mann-Whitney U Test

AIP type

Ninety-six patients were diagnosed with type 1 AIP (90%) and 11 patients with type 2 (10%; 3 definitive, 8 probable). Elevated serum IgG4 was found in 81 patients (84% of all patients, 90% of type 1 AIP). Thirty-four patients were operated for suspected pancreatic cancer (32%), of which 18 underwent a pancreatic resection (17%). Pancreatic histology was obtained in 29 patients (27%), either surgically (n=24) or by biopsy during endoscopic ultrasonography (n=5). Lymphoplasmacytic sclerosing pancreatitis (type 1 AIP) was found in 23 patients and idiopathic duct-centric pancreatitis (type 2) in three. As compared to type 1, the type 2 AIP group was younger (p<0.001), with less male predominance (p=0.036). Surprisingly, other organ involvement was not only found in type 1, but also in type 2 patients (70% and 45%, respectively). An association with inflammatory bowel disease was only observed in type 2 patients (36%).

AIP treatment and response

Steroid therapy was instituted in 89/107 patients (83%), all of which responded favourably. Sixty-five patients were treated with an initial dose of 30-40 mg prednisolone daily (78%). Three patients received a higher dose of 45-60 mg (3%) and 16 a low dose of 10-20 mg per day (18%). In 16 patients, azathioprine was added as (steroid sparing) maintenance therapy.

Fifty-five patients experienced a relapse (52%; Figure 2); 22 a single relapse (21%) and 34 multiple relapses (34%). Of note, relapses not only occurred in type 1 patients (55%), but also in 27% of type 2 patients. However, in type 2 disease, relapses never occurred more than once.

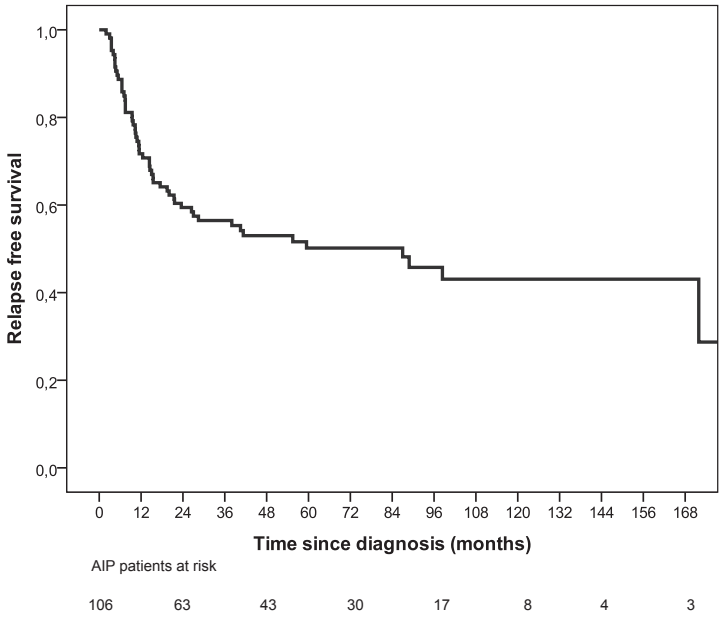

Figure 2. Relapse-free survival in AIP patients.

The first relapse occurred a median of 31 months (IQR 11-85) after diagnosis and most frequently involved the pancreas or biliary tract (55 and 67%, respectively; Figure 3). Furthermore, almost 80% of the relapses occurred within 2 years after diagnosis. All relapses were successfully treated with a restart of steroid therapy and in 28 patients, azathioprine was added. None of the evaluated items was associated with relapse (age, gender, presenting symptoms, other organ involvement, AIP type, serum IgG4 levels, diffuse pancreatic enlargement, steroid therapy or dosing, and pancreatic resection).

Survival

In total, 18 patients had died after a median of 70 months (IQR 47-120). Five of these patients died from complications of IgG4-RD, a median of 5 years after diagnosis (IQR 4-10; Figure 1). Two of these patients had declined steroid therapy, resulting in end-stage liver cirrhosis. The other three patients had a substantial diagnostic delay (median 4 years), and developed irreversible liver (n=2) or renal failure (n=1). Another patient with a substantial diagnostic delay also developed liver and renal organ failure, but this patient survived after a combined liver-kidney transplant. There was no significant difference in survival of AIP patients, as compared to an age- and sex-matched Dutch reference population (Figure 4).

Prospective outcome data

Of the 82 living patients, 68 agreed to participate (83%), 62 with type 1 AIP and 6 with type 2 (91% and 9%, respectively). Due to the small number of type 2 AIP patients, we were not able

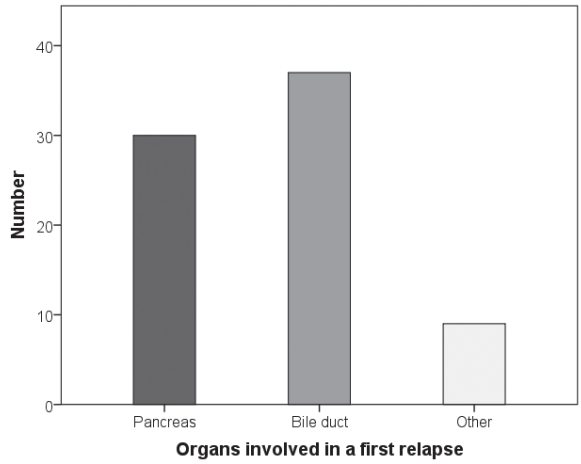

Figure 3. Distribution of organs involved in first relapse (n=55). Other organs included salivary glands (3), retroperitoneal fibrosis (4), prostate (1), kidney (1) and IBD (2). Simultaneous relapses in pancreas and bile duct occurred in 16 patients; in pancreas, bile duct and salivary glands in 1 patient; and in bile duct and retroperitoneum in 1 patient.

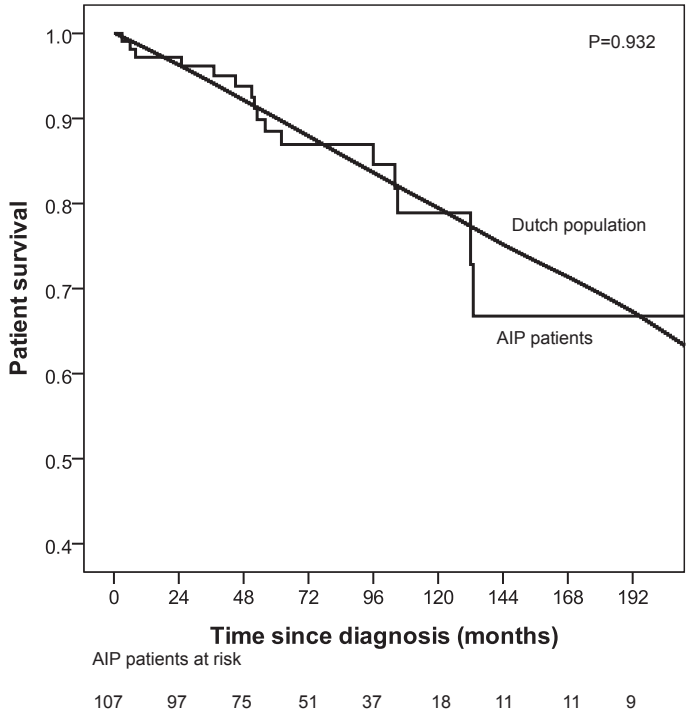

Figure 4. Survival of AIP patients, compared to an age- and sex-matched Dutch reference population. Log-rank test *P* value is reported.

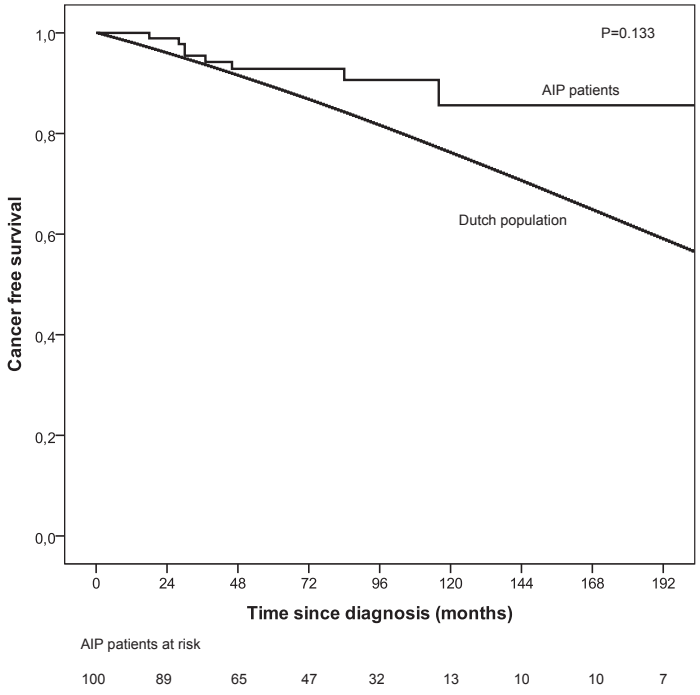

Figure 5. Cancer-free survival of AIP patients, compared to an age- and sex-matched Dutch reference population. Log-rank test *P* value is reported.

to perform a statistical analysis comparing type 1 and 2 AIP. The median follow-up of this group was 75 months (IQR 50-106). There were no significant differences in baseline characteristics between the patients who did and did not agree to participate (data not shown). Due to the small number of type 2 patients, statistical analysis, comparing the two AIP types, could not be performed. Thirty-one patients still received active treatment for AIP (46%); 11 prednisolone (35%), eight azathioprine (26%), 11 a combination of the two (35%), and one mercaptopurine (3%). During the study period, none of the patients developed pancreatic cancer. Eight patients developed some other type of cancer (8%); prostate (n=2), esophagus, colon, bladder, and lung cancer, leukemia and non-Hodgkin lymphoma. However, the general cancer risk of AIP patients was not different from an age- and sex-matched Dutch reference population (Figure 5).

Fifty-six patients were found to be exocrine insufficient (82%), of which only 32 had been diagnosed prior to the study and were already treated with enzyme supplementation (57%, Table 2). Exact logistic regression analysis revealed older age to be the only associated risk factor (Table 3; OR: 1.05 (95%CI: 1.0-1.1)). Endocrine insufficiency was present in 37 patients (57%), of which 6 were newly diagnosed by tests carried out as part of the study protocol (16%). Risk factors, associated with endocrine insufficiency, were a longer follow-up period (OR: 1.36 (95% CI: 1.11-1.68)) and older age (OR: 1.06 (95% CI: 1.01-1.11)). Importantly, there was no difference in pancreatic function for patients, who did and did not receive steroid therapy.

Table 4 shows the mean QoL scores, as compared to age- and sex-matched general population norms. None of the SF-36 component scores or QLQ-C30 subscales was lower in AIP patients.

Table 2. Prospective outcome; pancreatic endocrine and exocrine function status

Variable	(n=68)
Exocrine insufficiency[¥] – no. (%)	56 (82)
Feces elastase in µg/gram feces – median (IQR)	0.015 (0.015-0.104)
Pancreatic enzyme suppletion – no. (%)	32 (57)
Endocrine insufficiency[¥] – no. (%)	37 (57) [†]
Fasting glucose in mmol/L – median (IQR)	5.6 (5.1-6.4)
HbA1c in mmol/mol Hb – median (IQR)	40 (37-44)
Medication use for endocrine insufficiency – no. (%)	31 (70)
Insulin	19 (61)
Oral medication	17 (55)

[¥] Endocrine insufficiency defined as fasting glucose level >7.0 mmol/L, HbA1c level >42 mmol/mol (6.0%), or using prescribed antidiabetic medication
[¥] Exocrine insufficiency defined as fecal elastase level of <200 µg/gram feces
[†] The denominator differs, because serum samples were not available in 2/68 patients (3%).

Table 3. Multivariate logistic regression analysis for factors associated with exocrine and endocrine pancreatic insufficiency

Variables	Exocrine insufficiency, OR (95% CI)	p-value	Endocrine insufficiency, OR (95% CI)	p-value
Length of follow-up	1.24 (0.97-1.58)	0.08	1.36 (1.11-1.68)	0.003*
Age at onset[¥¶]	1.05 (1.01-1.09)	0.03*	1.06 (1.01-1.11)	0.02*
Male sex[¥]	3.41 (0.08-14.94)	0.10	1.66 (0.39-7.14)	0.49
Type 1 AIP[¥]	5.47 (0.92-32.66)	0.06	5.84 (0.70-48.85)	0.10
Pancreatic resection[¥]	5.50 (0.28-108.11)	0.26	3.09 (0.59-16.16)	0.18

[¥]Adjusted for length of follow-up; [¶]1-year increment; *Significant at p<0.05 level.

Table 4. Prospective outcome; Quality of life scores, as compared to age- and sex-matched general population norms

	AIP patients (n=67)	General Population Norms	*p*-Value'
SF-36¶			
Physical health component	48 ± 9	46	0.04*
Mental health component	53 ± 9	51	0.09
QoL QLQ-C30#			
Global health status	78 ± 17	77	0.61
Physical functioning	85 ± 17	88	0.21
Role functioning	87 ± 20	88	0.64
Emotional functioning	85 ± 20	90	0.06
Cognitive functioning	91 ± 12	90	0.65
Social functioning	91 ± 19	93	0.29

*p-value < 0.05, 'One-Sample T-test
¶The scores of the SF-36 component scores range from 0 to 100, with higher scores indicating better quality of life. Linear transformations were performed to standardize the scores to a mean score ± standard deviation of 50 ± 10 in a general Dutch population.
#Scores of the QLQ-C30 are linearly transformed to a scale of 0 to 100, with higher score indicating better functioning or global QoL.

DISCUSSION

This study is the first to evaluate the full range of long-term consequences of autoimmune pancreatitis, with a unique follow-up of more than 6 years. The results show that, despite a successful primary treatment response, more than half of the patients developed one or more relapses, and almost half of the patients required chronic maintenance therapy. None of the AIP patients developed pancreatic cancer during the study period, but most became both exo- and endocrine insufficient. Remarkably, AIP did not have a negative impact on quality of life or survival.

In this cohort, the vast majority of patients became both endocrine and exocrine insufficient over the years. Therefore, we recommend active screening for pancreatic insufficiency in AIP, especially in older patients, as age was identified as a risk factor. The observed prevalences correspond with reports from Japan, but are higher than numbers found in a European cohort.[10,15-17] This difference may be explained by the prospective nature of the present study, because a significant number of patients were diagnosed by tests performed as part of the study protocol. In addition, the long follow-up time (exceeding 6 years) might have contributed, as duration of follow-up was found to be a predictive factor for the development of pancreatic endocrine insufficiency.

Several studies have reported improvement of the pancreatic function after steroid therapy.[15,17-19] However, our data do not indicate that treatment can preserve the pancreatic function on the longer term. Prospective studies are needed to show if long-term maintenance therapy influences pancreatic insufficiency rates.

To our knowledge, the impact of AIP on quality of life was never studied before. Given the diagnostic difficulties and confusion with pancreatic cancer, it seemed reasonable to assume that AIP might have a negative long-term influence. However, in this cohort, the long-term quality of life was not impaired, even though almost half of the patients still used immunosuppressive therapy and most suffered from some form of pancreatic insufficiency. Again, the long follow-up period might have played a role, with the negative impact of AIP-related events waning over time. Also, conversion from the life-threatening diagnosis of pancreatic cancer to a benign and treatable disease may have resulted in a lasting sense of relief. Finally, in patients who experience a serious and possible life-threatening disorder, the response shift phenomenon is known to occur, meaning that changes in health status alter the internal standards of quality of life.[20]

All patients in this study responded favorably to steroid treatment. These excellent response rates have been reported many times before.[10,21-24] This implies that in patients who do not respond to steroid therapy, the diagnosis of AIP should be reconsidered. The value of steroid treatment is emphasized by the six patients who developed end-stage organ failure (of whom five died). None received steroid treatment (in time). Therefore, their uniform negative outcome may be seen as evidence of an unfavorable natural disease course. Previously, Sah et al described that AIP does not affect the long-term survival and we conclude the same.[25] However, our data show that this only holds true for patients, diagnosed and treated in a timely fashion, and that AIP is a potentially life-threatening disorder when patients are deprived of steroids.

Relapses were frequently observed, similar to previous reports.[22,24,25] We could not identify any risk factors for disease relapse. Importantly, we found no differences in relapse rate between patients, initially treated with a low or a high dose of prednisolone. However, only a small group of patients received a low dose, so further studies are needed to confirm these findings.[26] Interestingly, almost all relapses occurred in the first 2 years after diagnosis. This implies that clinicians should observe patients carefully in the first years after tapering steroids.

At follow-up, almost half of the patients still required treatment for AIP. This shows that, despite a successful initial treatment response, recurrences are common and many patients are unable to cease immunosuppressive therapy. Low-dose steroids are often used for maintenance. However, long-term steroid treatment may result in significant side effects, especially in an elderly population. As an alternative, immunomodulators, such as azathioprine, can be prescribed. Although some studies reported treatment-limiting side effects, others describe successful use, without serious complications, which is in line with our experience.[10,21,27-29]

Future studies are required to further define the role of azathioprine. Recently, rituximab has been suggested as an effective alternative for patients with difficult-to-treat, relapsing AIP, but this requires further investigation.[27]

The observed clinical profiles of type 1 and 2 disease were in correspondence with earlier findings, with two exceptions; First, in contrast to previous studies, we found other organ involvement to be quite common in type 2 patients.[10,24,25] In addition, previous studies reported a lower relapse rate in type 2 AIP patients, but in this study population, relapse rates were similar, although type 2 patients never relapsed more than once.[24,25] Perhaps, the much longer follow-up period of the present study allowed more time for relapses to develop, even in the

type 2 group. Unfortunately, a formal comparison between the two AIP types was impossible, due to the small number of type 2 patients. Of note, the proportion of type 2 patients in our cohort is similar to others.[10,24]

Several case reports suggested an increased risk of pancreatic cancer in AIP, similar to other forms of pancreatitis.[30-33] This was also suggested by Gupta et al., who examined histological specimens of operated patients with AIP and chronic pancreatitis, and concluded that pre-neoplastic ductal lesions were highly prevalent in both groups.[7] In contrast, in the present study, none of the patients developed pancreatic cancer. This corresponds with findings from another international cohort, which showed that the occurrence of pancreatic cancer following AIP was rare.[24] Given this conflicting evidence, additional prospective studies are needed to clarify this issue.

Our study comprises one of the largest AIP cohorts, especially in comparison to other European studies, and is the first to prospectively assess the pancreatic function and quality of life. Furthermore, the long follow-up period is unique and offers new insights in the long-term outcome of AIP. However, the study also has limitations. Some of the outcome data were collected retrospectively. Also, not all patients agreed to participate in the prospective part of the study. However, a selection bias is unlikely, given the size and characteristics of this group. In addition, patients were not treated according to a standard treatment protocol, as validated guidelines on this topic are lacking.

Ideally, only one criteria scoring system would have been used, preferably the recently proposed ICDC.[2] These criteria are a first step towards uniform diagnostic criteria for AIP, replacing national criteria, like the HISORt criteria. However, a previous study, focusing on the diagnostic performance of the AIP scoring systems, showed their complementary rather than overlapping use.[8] For type 1 AIP patients, when imaging is not available or shows no pancreatic abnormalities, the HISORt criteria are the only criteria that can establish an AIP diagnosis, based on positive serology and response to steroids. On the other hand, if IgG4 levels are normal, AIP cannot be diagnosed by the HISORt criteria. In type 2 AIP patients, it is nearly impossible to establish a diagnosis non-histologically, mainly because of the less-known clinical features of type 2 patients and the lack of a serological marker, as described by Ikeura.[9] In our view, this might lead to the misclassification of type 2 as type 1 patients. Therefore, we chose to use the system proposed by Maire et al.[10]

In summary, in this large cohort of AIP patients we show that recurrences are common, despite a successful initial treatment response (even in type 2 patients), and almost half of the patients require long-term maintenance therapy. Pancreatic cancer was not observed, but pancreatic insufficiency was extremely common. Therefore, regular evaluation of the pancreatic function is highly recommended, especially in older patients. Long-term quality of life and (cancer free) survival are not impaired.

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CHAPTER 9

SUMMARY AND GENERAL DISCUSSION

INTRODUCTION

Autoimmune pancreatitis (AIP) is a novel type of chronic pancreatitis, which is increasingly being recognized. There are two different subtypes. Type 1 represents the pancreatic manifestation of a systemic, IgG4-related disease (IgG4-RD), which can involve other organ systems. It is the most common form and relapses are frequently observed. Type 2 is pancreas-specific, not associated with elevated serum IgG4, and rarely causes relapses. Clinically, AIP mimics pancreatic cancer, with painless obstructive jaundice and weight loss as most common symptoms. Unlike other types of pancreatitis, AIP responds dramatically to steroid therapy. A timely diagnosis is of utmost importance, to provide proper treatment and avoid unnecessary interventions and complications.

Chapter 1 provides an introduction. Here, we discuss the clinical profile of AIP and give an overview on current treatment options. Furthermore, we present the aims and outline of this thesis; to study the diagnostic process, treatment, and outcome of this disease.

MAIN FINDINGS

AIP cohort

To do so, in **chapter 2,** we describe the characteristics of a group of 114 AIP patients, retrieved from a prospectively maintained database that was established by three tertiary referral centers (Rotterdam, Amsterdam, Utrecht), in cooperation with several other hospitals. The median age of these patients was 62 years and 87% were men. The most common presenting symptoms were obstructive jaundice (76%) and weight loss (88%). Furthermore, mild abdominal pain (54%) and signs of endocrine and exocrine pancreatic insufficiency (35% and 75%, respectively) were reported frequently.

Diagnostic scoring systems

As the clinical presentation of AIP resembles pancreatic carcinoma, establishing the diagnosis can be challenging. Several diagnostic scoring systems have been proposed that combine radiological, histological, and serological evidence of AIP, other organ involvement, and response to therapy[1-3]. The most commonly used are scoring systems from the United States (HISORt) and Asia (Asian criteria). Recently, an international panel of experts developed a new set of criteria, the International Consensus Diagnostic Criteria (ICDC), which combines features from the HISORt and Asian criteria.

In **chapter 2**, we evaluate the performance of these three diagnostic scoring systems in our AIP cohort. These scoring systems were applied retrospectively, using data obtained during the original diagnostic phase, to mimic clinical practice. Although the majority of patients fulfilled the requirements of one or more scoring systems, 21 (18%) did not, even though these patients had an unchallenged diagnosis of AIP. The systems proved to be complementary, rather than overlapping. In accordance with previous reports, the best result was achieved by the ICDC, with 68% of patients fulfilling the requirements, followed by the HISORt (52%) and Asian criteria (33%)[4-6].

The ICDC are the only criteria distinguishing type 1 and 2 AIP. However, as the definition of type 2 is based solely on histology, which is not always available, this can lead to under-recognition of type 2. Furthermore, their clinical applicability is limited, due to their complexity. Based on these facts, we primarily recommend the use of the HISORt criteria, and advice to use the Asian criteria, if a pancreatogram is available. If the diagnosis cannot be confirmed by either of these systems, the ICDC can be used. Further studies should evaluate the specificity of the diagnostic criteria, to assess the risk for false-positivity.

Diagnostic markers

As AIP is difficult to distinguish from pancreatic cancer, reliable diagnostic markers are desperately needed. In **chapter 3,** we report the diagnostic value of the tumor marker Ca19-9, in differentiating AIP from other pancreatobiliary disorders. We compared serum samples from 33 patients with AIP, 53 with pancreatic carcinoma, and 145 with other pancreatobiliary disorders. Indeed, we found that patients with AIP have lower levels of Ca 19-9 and higher levels of IgG4 than those with pancreatic carcinoma. However, the diagnostic value of Ca19-9 and IgG4 alone was limited, because a marked overlap exists between the two disorders.

Our study showed that high levels of Ca19-9 (ranging from 5000-23000 U/ml) did not rule out AIP. Furthermore, elevated Ca19-9 levels were almost twice as common in AIP patients than previously reported. Although, individually, neither test was accurate enough to distinguish AIP from pancreatic cancer, the two tests combined reached a sensitivity of 94% and a specificity of 100%. This emphasizes the importance of combined Ca19-9 and IgG4 testing, when AIP is considered. Currently, the IgG4 upper limit of normal is set on 1.4 g/L, even though our data (in line with other reports) show that only levels above 2.6 g/L are 100% specific for AIP[7]. To prevent pancreatic carcinoma patients with mildly elevated IgG4 levels to be misdiagnosed as AIP, we suggest raising this upper limit of normal.

Elevated serum IgG4 is one of the main characteristics of AIP and is incorporated in all diagnostic scoring systems. However, particular caution is warranted in interpreting elevated levels of IgG4, since they are also encountered in other disorders, including chronic pancreatitis and primary sclerosing cholangitis. In **chapter 4**, we compared serum IgG4 levels in patients with acute, chronic, and autoimmune pancreatitis. In total, samples from 174 patients were evaluated; 32 with AIP, 90 with acute pancreatitis (AP) and 52 with chronic pancreatitis (CP). Our results showed that elevated IgG4 levels occurred in almost one out of ten AP patients and one out of five CP patients. However, in AP and CP, IgG4 levels rarely exceeded twice the upper limit of normal. Therefore, this study emphasizes increasing the cut-off value to diagnose AIP as well. We found that none of the other IgG subclasses could compete with IgG4 as individual predictor of AIP. However, in mildly elevated IgG4 (1-2x ULN), the IgG4/IgG2 ratio can substantially improve the positive predictive value.

In 2009, an Italian study identified a novel serologic marker, anti-PBP antibodies, with an outstanding sensitivity and specificity for diagnosing AIP, described in the New England Journal of Medicine[8]. We aimed to validate these results in our own cohort of AIP patients in **chapter 5**. To do so, we set up an ELISA assay, which sensitively and reliably measured reactivity against the AKEERRY peptide. Subsequently, we tested 34 patients with AIP, 29 with pancreatic carcinoma,

17 with CP, 16 with primary sclerosing cholangitis, 9 *H.pylori* negative healthy controls and 9 *H.pylori* positive healthy controls. However, no significant difference in detection of antibodies against the PBP peptide was found among the different patient groups or healthy controls. Therefore, we concluded that this is not a useful diagnostic tool to diagnose AIP.

IgG4-related disease and its pathogenesis

Type 1 AIP represents the pancreatic manifestation of a systemic, IgG4-related disease. Other organs are involved in approximately half of the patients and, up to now, a wide range of organ-localizations has been reported, from biliary to prostatic involvement. Other organ involvement can precede, coincide or follow the presence of pancreatitis. In contrast to other autoimmune disorders, patients with IgG4-RD are typically male and over 50 years old. Mostly, IgG4-RD has a subclinical presentation with mild symptoms, and is diagnosed coincidentally by the radiologist. However, it may cause severe symptoms and even organ failure[9,10].

In our AIP cohort, we identified nine patients with IgG4-related prostatitis. In **chapter 6**, we described their clinical and histological characteristics, and compared the presence of IgG4-RD features in prostatic tissue with 18 prostatitis control patients. This showed that infiltration of IgG4 positive plasma cells and an increased number of eosinophils were indicative for IgG4-related prostatitis. In two patients, prostatic symptoms improved after steroid therapy, which was given for AIP. Therefore, we advise to actively look for these features, to identify a subgroup of prostatitis patients, for whom steroid therapy may be beneficial.

The pathogenesis of IgG4-RD is still poorly understood and is most likely multifactorial. Given the infiltration of plasma cells in the affected tissue, and the excellent response to steroid therapy, an immune-mediated disorder was soon considered. A likely mechanism is that the disease develops in genetic susceptible persons, after exposure to certain triggers.

Whether serum IgG4 is pathogenic or an innocent bystander of the inflammatory response in IgG4-RD remains unknown. There is no linear association between symptoms and the level of serum IgG4. Also, serum IgG4 may remain elevated after therapy and resolution of symptoms, which suggests only a secondary role of IgG4. Furthermore, there are other clinical conditions associated with elevated serum IgG4, in which a protective effect is suspected, such as maintenance of food tolerance in atopic individuals and patients who underwent bee-venom immunotherapy[11,12].

However, a recent study from Amsterdam demonstrated that in patients with IgG4-related autoimmune cholangitis (IAC), IgG4-positive B-cell clones are abundantly present in both serum and inflamed tissue, compared to healthy and disease controls[13]. This suggested an antigen-driven immune response. In search for a causative agent leading to chronic antigenic stimulation, they noticed that the majority of their cohort had a history of blue collar work, which was validated in a second, independent cohort[14]. Chronic exposure to toxic dusts, industrial oils and paint for example could therefore provide an environmental trigger. Further support for the role of B-cells in the pathogenesis of IgG4-RD, is the swift improvement of symptoms after B-cell depletion therapy with rituximab in patients refractory to steroid therapy[15].

Furthermore, in a recent British study, serum IgG4 antibodies in patients with melanoma have been described to promote tumour progression, by contributing to a defective anti-

tumour immune response[16]. Chronic exposure to tumor antigens is suggested to induce this extensive IgG4 antibody production by B-cells, and elevated levels of serum IgG4 appear to be a negative predictor of progression-free and overall survival in these patients[17]. The authors further suggest that this knowledge might contribute to improved patient stratification and optimal personalized therapies.

Treatment

The first therapeutic step in AIP is to induce clinical remission with steroids. Patients with AIP show a dramatic response to steroid therapy, with response rates of nearly 100%. Currently, the recommended induction dosage is 30-40mg/day, however, this is largely based on empirical data and lacks a scientific basis. Whether a lower dose would evoke the same response is unclear, but could potentially prevent patients from the well-known side effects of high dose steroids. Therefore, in **chapter 7**, we retrospectively compared the efficacy of dosages ranging from 10 to 60 mg/day. Surprisingly, we found clinical, biochemical, and radiological responses to be comparable. These results await confirmation, ideally by controlled trials comparing the efficacy and tolerance of low- and high-dose induction corticosteroid therapy, although such a study would be difficult to perform.

Although remission induction is easy in AIP, treatment may be complicated, because recurrences are common and maintenance therapy may be necessary in some patients. After the first relapse, azathioprine is often added, after which steroids are tapered. However, some patients report treatment-limiting side effects and further studies are needed to define the role of azathioprine as maintenance therapy. Recently, rituximab has been suggested as an effective alternative for patients with difficult-to-treat, relapsing AIP, but this requires further investigation[18].

Long-term outcome

Since AIP is a newly recognized disease entity, little is known about the long-term consequences. In **chapter 8,** we study the long-term outcome of AIP, in terms of of treatment response, pancreatic function, quality of life, risk of pancreatic cancer, and mortality. We found that, despite an initial excellent treatment response, recurrences were common and almost half of AIP patients required long-term maintenance therapy.

Furthermore, the prevalence of exocrine and endocrine insufficiency in our cohort was striking, 82% and 57%, respectively. Pancreatic insufficiency in AIP patients has been described before, but our prevalences were much higher than earlier reported[19,20]. This might be explained by the prospective nature of our study, as a significant number of patients was diagnosed by tests carried out as part of the study protocol. This suggests that pancreatic insufficiency is significantly under-diagnosed in AIP patients. We therefore recommend active screening for pancreatic insufficiency, as this can result in severe complications.

Surprisingly, AIP did not have a negative impact on the quality of life. Given the diagnostic difficulties and confusion with pancreatic cancer, it seemed reasonable to assume that AIP might have a negative long-term influence. However, although almost half of the patients still used immunosuppressive therapy and most suffered from pancreatic insufficiency, the long-term quality of life was not impaired.

Finally, we also evaluated the risk for pancreatic cancer and the impact on mortality. Other forms of pancreatitis harbour an increased risk for pancreatic cancer, and the same association has been reported for AIP[27,28]. However, none of the patients in our cohort developed pancreatic cancer and the general cancer risk was not different from an age- and sex-matched Dutch reference population. Possibly related, survival of AIP patients, compared to a reference population, was not impaired either. Future follow-up studies are needed to confirm our findings.

CONCLUSIONS AND FUTURE PROSPECTIVES

Although in the past decade, clinical features and treatment of AIP and IgG4-RD have been further elucidated, the pathogenesis remains largely unclear. Different roles for IgG4 have been suggested, from pathogenic to that of an innocent bystander. A better understanding of the pathogenesis could lead to more targeted therapies.

Diagnostic markers are still urgently needed, especially to differentiate AIP from pancreatic cancer and to prevent redundant investigations or even major surgery. Regarding AIP and IgG4-RD treatment strategies, prospective randomized studies are needed to generate evidence-based data. However, these studies will be difficult to complete, as this is a rare disease and large numbers of patients are needed. For this reason, international collaborations are crucial.

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APPENDIX

NEDERLANDSE SAMENVATTING
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NEDERLANDSE SAMENVATTING

Introductie

De alvleesklier ('pancreas' in het Latijn) is een worstvormig orgaan dat zich bevindt in de buikholte, net achter de maag. Het heeft twee belangrijke functies; het produceren van spijsverteringssappen en de regulatie van de bloedsuikerspiegel, door afgifte van hormonen. De alvleesklier kan ontstoken raken, hetgeen 'pancreatitis' genoemd wordt. Dit proefschrift gaat over een zeldzame vorm van alvleesklierontsteking, namelijk autoimmuun pancreatitis (afgekort 'AIP'). Pas de laatste 15 jaar is er meer aandacht gekomen voor dit type pancreatitis, waarvan de oorzaak nog onbekend is.

AIP heeft twee verschillende subtypes: type 1 en type 2. Type 1 komt het meest voor en is vaak onderdeel van een systeemziekte, die zich in verschillende organen kan presenteren. Deze ziekte wordt ook wel 'IgG4-related disease' genoemd, omdat het zich kenmerkt door een verhoogd gehalte van het antilichaam IgG4. Type 2 betreft alleen de alvleesklier en gaat niet samen met een verhoogd IgG4 gehalte.

Patiënten met AIP ontwikkelen vaak geelzucht en gewichtsverlies. Deze klachten lijken op die van alvleesklierkanker, waardoor het voor artsen lastig is beide ziektebeelden van elkaar te onderscheiden. Echter, er is een belangrijk verschil; AIP is goed te behandelen met medicijnen, terwijl alvleesklierkanker meestal dodelijk is en alleen kan worden genezen door een operatie. Het is daarom extreem belangrijk om onderscheid te maken tussen de twee aandoeningen.

Bijna alle patiënten met AIP reageren goed en snel op de behandeling met ontstekingsremmende medicijnen, ook wel steroïden genoemd. Deze behandeling wordt meestal weer gestopt als de ziekteverschijnselen zijn verdwenen. Bij een deel van de patiënten (met name met type 1 AIP) komen de klachten echter weer terug, waarvoor een onderhoudsbehandeling wordt voorgeschreven.

Om onderzoek te kunnen doen naar dit ziektebeeld hebben wij een grote hoeveelheid gegevens van AIP patiënten verzameld in een database. Hierin zijn allerlei eigenschappen van patienten vastgelegd, zoals de klachten waarmee ze zich presenteerden en de afwijkingen die zij hadden bij aanvullend onderzoek. Daarnaast hebben we ook de reactie op behandeling en de lange termijn gevolgen bijgehouden.

Belangrijkste resultaten

In **hoofdstuk 1** worden de doelen en de inhoud van dit proefschrift besproken. Daarnaast wordt een algemeen overzicht gegeven van AIP, met onder andere de symptomen, behandelingsmogelijkheden en eventuele onderwerpen voor toekomstig onderzoek.

Het stellen van de diagnose AIP is moeilijk. Er zijn verschillende combinaties van diagnostische criteria bedacht om AIP te kunnen onderscheiden van andere alvleesklieraandoeningen. In **hoofdstuk 2** vergelijken we de bruikbaarheid van drie van deze samengestelde criteria in een cohort van 114 AIP patiënten. Hoewel in 82% de diagnose met één of meerdere systemen bevestigd kon worden, gold dit niet voor de overige 18% van de patiënten; zij voldeden aan geen van de drie definities.

Omdat de symptomen van alvleesklierkanker en AIP gelijk zijn, zou een onderscheidende test, bij voorkeur een simpele bloedtest (serum marker), heel waardevol zijn. In **hoofdstuk 3**

onderzoeken we twee serum markers: Ca19-9 en IgG4. Ca 19-9 is een tumormarker, die vaker verhoogd is bij alvleesklierkanker, en IgG4 is een antilichaam dat vaak verhoogd is bij AIP. Zoals we verwachtten, hadden AIP patiënten een hoger IgG4 gehalte en een lager Ca 19-9 dan patiënten met alvleesklierkanker. Geen van deze tests bleek echter betrouwbaar genoeg om AIP te onderscheiden van alvleesklierkanker. Wanneer beide tests gecombineerd werden daarentegen, maakten ze wel voldoende onderscheid.

In **hoofdstuk 4** hebben we IgG4 bloedspiegels vergeleken van patiënten met verschillende vormen van alvleesklier ontsteking: acute, chronische en autoimmuun pancreatitis. Ondanks dat een verhoog IgG4 een belangrijk kenmerk van AIP is, bleek dit ook voor te komen bij één op de tien patiënten met acute pancreatitis en één op de vijf patiënten met chronische pancreatitis. IgG4 waardes die meer dan 2x verhoogd waren, kwamen maar zelden voor bij andere aandoeningen dan AIP. Licht verhoogde IgG4 waardes moeten dus zorgvuldig geïnterpreteerd worden om niet de verkeerde diagnose te stellen. Daarnaast zou ons advies zijn, om de afkapwaarde van een normale IgG4 waarde te verhogen.

In 2009 werd in een Italiaanse studie een nieuwe marker voor AIP beschreven, anti-PBP antilichamen, die zeer nauwkeurig leek te zijn. In **hoofdstuk 5** beschrijven we een laboratorium opzet, waarmee we betrouwbaar reactiviteit tegen PBP antilichamen meten in ons eigen cohort van AIP patiënten. Er werden echter geen verschillen in reactiviteit gevonden tussen de verschillende patiënten groepen. Deze test blijkt daarmee onbruikbaar om AIP te diagnosticeren.

In **hoofdstuk 6** beschrijven we een reeks patiënten met type 1 AIP, bij wie de systeemziekte ook in de prostaat voorkomt. We hebben prostaat weefsel van deze patiënten vergeleken met weefsel van een controlegroep met een ander type prostaatontsteking. Wat opviel, is dat een aantal kenmerken vaker voorkwam bij AIP patiënten, namelijk IgG4-positieve cellen en een verhoogd aantal eosinofielen (een speciaal type witte bloedcellen).

Patiënten met AIP reageren in het algemeen goed op een behandeling met steroïden. Hoewel er geen wetenschappelijk onderzoek gedaan is naar welke dosis het beste werkt, wordt in de praktijk meestal 30-40 milligram per dag gegeven. Aangezien steroïden forse bijwerkingen kunnen geven, onderzochten wij in **hoofdstuk 7** of een lagere dosering net zo effectief is. We concludeerden dat de reactie op deze behandelingen vergelijkbaar was.

In **hoofdstuk 8** hebben we de gevolgen van AIP op de langere termijn onderzocht. Omdat AIP relatief kort geleden als aparte ziekte werd ontdekt, is hierover nog weinig bekend. Wij kwamen tot de conclusie dat, ondanks een goede eerste reactie op behandeling, de ziekte vaak terugkomt, en bijna de helft van de patiënten een onderhoudsbehandeling nodig heeft. In ons cohort heeft niemand alvleesklierkanker ontwikkeld, maar bleek de functie van de alvleesklier bij meer dan driekwart van de patiënten sterk verminderd. Hun kwaliteit van leven en overleving was echter niet anders dan van de gemiddelde Nederlandse bevolking.

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Presentations at international conferences

Diagnostic scoring systems for autoimmune pancreatitis are complementary and correctly identify the majority of patients at initial presentation, without the need for histology
Poster presentation, Digestive Disease Week, San Diego, USA, 2012
Poster presentation, United European Gastroenterology Week, Amsterdam, The Netherlands, 2012

Comparable efficacy of low and high dose induction corticosteroid treatment in autoimmune pancreatitis
Poster presentation, Digestive Disease Week, San Diego, USA, 2012
Poster presentation, United European Gastroenterology Week, Amsterdam, The Netherlands, 2012

IgG4-related prostatitis in patients with autoimmune pancreatitis
Poster presentation, Digestive Disease Week, Orlando, USA, 2013
Poster presentation, United European Gastroenterology Week, Berlin, Germany, 2013

The long-term outcome of autoimmune pancreatitis
Oral presentation, United European Gastroenterology Week, Berlin, Germany, 2013
Poster presentation, Digestive Disease Week, Orlando, USA, 2013

Serum IgG4 in acute, chronic and autoimmune pancreatitis
Oral presentation, United European Gastroenterology Week, Vienna, Austria, 2014
Poster presentation, Digestive Disease Week, Washington, USA, 2015

Testing for anti-PBP antibodies is not usefull in diagnosing autoimmune pancreatitis
Poster presentation, Digestive Disease Week, Washington, USA, 2015

Presentations at national conferences

Comparable efficacy of low and high dose induction corticosteroid treatment in autoimmune pancreatitis
Oral presentation, Dutch Society of Gastroenterology, The Netherlands, 2012

The long-term outcome of autoimmune pancreatitis
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Good Clinical Practice (BROK) course
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Peer review activities

Journal of Clinical Gastroenterology
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Buijs J, van Heerde MJ, van Buuren HR, Bruno MJ, Cahen DL.
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van Heerde MJ, Bakker-Jonges L, Hansen BE, van Ettinger K, Gilbert M, Batstra M, van Toorenenbergen A, de Waart M, Hooijkaas H, Dufour-van den Goorbergh D, van Vuuren H, Francke J, Heijens A, van Eijck CHJ, Kazemier G, Pek C, Poley JW, **Buijs J**, Bruno MJ, Kuipers EJ, van Buuren HR.
Testing for autoantibodies including antinuclear antibody, rheumatoid factor, anti carbonic anhydrase II and anti lactoferrin is not useful in diagnosing autoimmune pancreatitis in a Western population
Submitted.

Buijs J, Cahen DL, van Heerde MJ, Hansen BE, van Buuren HR, Peppelenbosch MP, Fuhler GM, Bruno MJ.
Testing for anti-PBP antibody is not useful in diagnosing autoimmune pancreatitis.
Submitted.

Buijs J, Bruno MJ, Cahen DL, van Heerde MJ, Hollemans RA, Hansen BE, Besselink MG, van Santvoort HC, van Buuren HR, Bruno MJ.
The value of elevated serum IgG4 and IgG4/IgG2 ratio in autoimmune, acute and chronic pancreatitis.
Submitted.

Buijs J, Cahen DL, van Heerde MJ, Rauws EAJ, Maillette de Buy Wenniger LJ, Hansen BE, Biermann K, Verheij J, Vleggaar FP, Brink MA, Beuers UHW, van Buuren HR, Bruno MJ.
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Digestive Diseases and Sciences 2014; 59(6):1322-9.

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Lieve Pieter, wat zijn we verschillend, maar wat zijn we een goed team! We hebben al heel wat mooie jaren achter de rug en ik kijk uit naar alle jaren die nog gaan komen!

CURRICULUM VITAE

Jorie Buijs werd geboren op 25 juni 1985 te Driehuizen. In 2003 behaalde zij haar eindexamen aan het Murmellius Gymnasium te Alkmaar. Zij studeerde gedurende één jaar Biomedische Wetenschappen aan de Vrije Universiteit te Amsterdam, alvorens in 2004 te starten met de studie Geneeskunde aan de Erasmus Universiteit Rotterdam. In 2008 behaalde zij haar doctoraal diploma, nadat ze haar afstudeeronderzoek afrondde in het Hospital del Niños in Lima, Peru. In 2010 doorliep zij haar keuze coschap Interne Geneeskunde in het Moi Teaching and Referral Hospital in Eldoret, Kenia en haar oudste coschap op de afdeling Maag-, Darm- en Leverziekten in het Erasmus Medisch Centrum, Rotterdam. Na het behalen van het arts-examen in april 2011 startte zij met promotieonderzoek op de afdeling Maag-, Darm- en Leverziekten van het Erasmus Medisch Centrum onder begeleiding van prof. M.J. Bruno, dr. H.R. van Buuren en dr. D.L. Cahen. Mei 2014 is ze gestart met de opleiding tot Maag-, Darm- en Leverarts (opleider dr. R.A. de Man). Op dit moment zit zij in het tweede jaar van haar vooropleiding Interne Geneeskunde (opleider dr. C.G. Vermeij) in het Deventer Ziekenhuis.